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0 5 OCI 2004



REC'D 0 5 JUN 2003
WIPO PCT

Kongeriget Danmark

Patent application No.:

PA 2002 00533

Date of filing:

10 April 2002

Applicant:

(Name and address)

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Title: Improved Bacillus Host Cell

IPC: -

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PRIORITY DOCUMENT

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Patent- og Varemærkestyrelsen Økonomi- og Erhvervsministeriet

29 April 2003

Pia Høybye-Olsen

PATENT- OG VAREMÆRKESTYRELSEN

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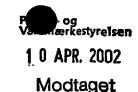
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TITLE: Improved Bacillus Host Cell

TECHNICAL FIELD

Bacillus sp. are attractive hosts for the production of heterologous proteins due their ability to secrete proteins directly into the culture medium. They have a high capacity for protein secretion, are genetically highly amenable, nonpathogenic and free of endotoxins, and consequently a large variety of proteins from different organisms have been efficiently produced and secreted in Bacillus sp. i.e. in Bacillus licheniformis.

In the highly competitive biotech industry, even slightly improved *Bacillus* host cells are in demand, which may provide more attractive production systems, or may even just be alternative production systems.

BACKGROUND

Many industrial products of commercial interest can be produced biologically in *Bacillus sp.* host cells e.g. heterologous polypeptides, amino acids, carbohydrates etc. Some of these products are sold as process aids, intermediates, or even end-products in the food and feed industries as well as in the pharmaceutical industry. There are increasingly strict regulations that must be complied with when producing such products in microbial production hosts for sale in these industries, for instance the presence of bacterial spores in the products is seen as a problem. When producing in *Bacillus licheniformis* it is thus desirable to ensure that the host cell is not capable of forming spores.

SUMMARY

A problem to be solved by the present invention is how to obtain a *Bacillus licheniformis* host cell incapable of forming spores, or how to impair the sporulation process of said cell. The present invention provides a solution to the problem by providing a *Bacillus licheniformis* host cell which has a reduced capacity to produce one or more polypeptide(s) involved in sporulation.

Accordingly, in a first aspect the invention relates to a *Bacillus licheniformis* mutant host cell derived from a parent *B. licheniformis* host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in sporulation which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 129, preferably at least 85% identical, more preferably at least 90% identical, still more preferably at least 95% identical, and most preferably at least 97% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 129, wherein the mutant host cell expresses at least 5% less of the one or more polypeptide(s) involved in sporulation than the parent host cell, when they are cultivated under comparable conditions. Preferably the mutant host cell expresses at

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least 10% less, more preferably at least 20% less, still more preferably at least 30% less, even more preferably at least 40% less, yet more preferably at least 50% less, or at least 60% less, or at least 80%, or most preferably at least 90% less of the one or more polypeptide(s) involved in sporulation than the parent host cell, when they are cultivated under comparable conditions. Most preferably the mutant host cell expresses absolutely nothing of the one or more polypeptide(s) involved in sporulation.

Comparable conditions of cultivation must be used in order to compare the expression level of the one or more polypeptide(s) involved in sporulation in a mutant host cell of the invention with that in a parent host cell. They are cultivated separately under identical conditions in identical setups, of course allowing for the usual standard deviations of the operating parameters normally associated with growth experiments, such as temperature control etc. The quantification of the expression level of the one or more polypeptide(s) is done by standard text-book assay techniques as known in the art e.g. mRNA quantification or immuno-based assays.

In a second aspect the invention relates to a process for producing at least **one** product of interest in a *Bacillus licheniformis* mutant host cell, comprising cultivating a *B.licheniformis* mutant host cell as defined in the previous aspect in a suitable medium, whereby the said product is produced.

Finally, an aspect of the invention relates to a use of a *Bacillus licheniformis* mutant host cell as defined in the first aspect for producing at least one product of interest comprising cultivating the mutant host cell in a suitable medium whereby the said product is produced.

DEFINITIONS

Nucleic acid construct: When used herein, the term "nucleic acid construct" means a nucleic acid molecule, either single- or double-stranded, which is isolated from a naturally occurring gene or which has been modified to contain segments of nucleic acids in a manner that would not otherwise exist in nature. The term nucleic acid construct is synonymous with the term "expression cassette" when the nucleic acid construct contains the control sequences required for expression of a coding sequence of the present invention.

Control sequence: The term "control sequences" is defined herein to include all components, which are necessary or advantageous for the expression of a polypeptide of the present invention. Each control sequence may be native or foreign to the nucleotide sequence encoding the polypeptide. Such control sequences include, but are not limited to, a leader,

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polyadenylation sequence, propeptide sequence, promoter, signal peptide sequence, and transcription terminator. At a minimum, the control sequences include a promoter, and transcriptional and translational stop signals. The control sequences may be provided with linkers for the purpose of introducing specific restriction sites facilitating ligation of the control sequences with the coding region of the nucleotide sequence encoding a polypeptide.

Operably linked: The term "operably linked" is defined herein as a configuration in which a control sequence is appropriately placed at a position relative to the coding sequence of the DNA sequence such that the control sequence directs the expression of a polypeptide.

Coding sequence: When used herein the term "coding sequence" is intended to cover a nucleotide sequence, which directly specifies the amino acid sequence of its protein product. The boundaries of the coding sequence are generally determined by an open reading frame, which usually begins with the ATG start codon. The coding sequence typically include DNA, cDNA, and recombinant nucleotide sequences.

Expression: In the present context, the term "expression" includes any step involved in the production of the polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

Expression vector: In the present context, the term "expression vector" covers a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of the invention, and which is operably linked to additional segments that provide for its transcription.

DETAILED DISCLOSURE

A Bacillus licheniformis mutant host cell derived from a parent B. licheniformis host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in sporulation which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 129, wherein the mutant host cell expresses at least 5% less of the one or more polypeptide(s) involved in sporulation than the parent host cell, when they are cultivated under comparable conditions.

The term "parent host cell" in the context of the present invention means a cell which is genetically identical, or isogenic, to the progeny mutant or mutant cell of the present invention, except for the mutated one or more gene(s) encoding one or more polypeptide(s) involved in sporulation in said mutant.

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The degree of identity, or %-identity of polypeptide sequences can suitably be investigated by aligning the sequences using a computer program known in the art, such as "GAP" provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711)(Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443–453). Using GAP with the following settings for DNA sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3".

An object of the present invention is to provide a culture medium free of bacterial spores so as to reduce the product purification to a minimum, and to comply with regulatory requirements. This may be done according to the invention by reducing or even completely abolishing the expression of one or more gene(s) encoding a native polypeptide(s) involved in sporulation via mutagenisation of that (those) gene(s). One of the very well-known method of ensuring that a gene is not expressed into an active polypeptide within a cell is simply to delete or partially delete the encoding gene. Many techniques have been described in the art on how to specifically delete or partially delete one or more gene(s) in the genome of a cell, and certainly from the genome of a *Bacillus licheniformis* cell (see e.g. Novozymes A/S WO 01/90393, Novozymes A/S WO 02/00907). Accordingly, a preferred embodiment of the present invention relates to a host cell of the first aspect, which is mutated by a partial or complete deletion of the one or more gene(s) encoding the one or more polypeptide(s) involved in sporulation.

A preferred embodiment of the present invention relates to a host cell of the first aspect, which is mutated in two or more genes encoding two or more polypeptides involved in sporulation.

The product of interest to be produced by the mutant host cell of the first aspect may be one or more polypeptide(s) encoded by one or more heterologous gene(s). Consequently, a preferred embodiment of the present invention relates to a host cell of the first aspect, which comprises one or more heterologous gene(s) encoding one or more heterologous polypeptide(s).

In the industrial production of polypeptides it is of interest to achieve a product yield as high as possible. One way to increase the yield is to increase the copy number of a gene encoding a polypeptide of interest. This can be done by placing the gene on a high copy number plasmid. However, plasmids are unstable and are often lost from the host cells if

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there is no selective pressure during the cultivation of the host cells. Another way to increase the copy number of the gene of interest is to integrate it into the host cell chromosome in multiple copies. Integration of two genes has been described in WO 91/09129 and WO 94/14968 (Novozymes A/S) the content of which is hereby incorporated by reference. A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) is present in at least two copies, preferably at least 4 copies, and most preferably at least 6 copies. In another embodiment the heterologous gene(s) is present in at least ten copies. If carried on a plasmid the gene(s) may be present in several hundred copies per cell, so in a still further embodiment of the present invention the heterologous gene(s) is present in at least 100 copies.

Integration of two genes closely spaced in anti-parallel tandem to achieve better stability has been described in WO 99/41358 (Novozymes A/S) the content of which is hereby incorporated by reference, as well as the stable chromosomal multi-copy integration of genes described in WO 02/00907 (Novozymes A/S) the content of which is incorporated herein by reference. A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) are stably integrated into the genome of the cell.

Selection of chromosomal integrant has for convenience resulted in the use of selectable markers such as antibiotic resistance markers. However it is desirable if possible to avoid the use of antibiotic marker genes. WO 01/90393 discloses a method for the integration of a gene in the chromosome of a host cell without leaving antibiotic resistance markers behind in the strain, the content of which is hereby incorporated by reference A preferred embodiment of the present invention relates to a host cell of the first aspect wherein the heterologous gene(s) is integrated into the genome of the cell without leaving any antibiotic resistance marker gene(s) at the site of integration.

The present invention also relates to nucleic acid constructs comprising a nucleotide sequence encoding a product of interest, which may be operably linked to one or more control sequences that direct the expression of the coding sequence in a suitable host cell under conditions compatible with the control sequences.

A nucleotide sequence encoding a polypeptide ofinterest may be manipulated in a variety of ways to provide for expression of the polypeptide. Manipulation of the nucleotide sequence prior to its insertion into a vector may be desirable or necessary depending on the expression vector. The techniques for modifying nucleotide sequences utilizing recombinant DNA methods are well known in the art.

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Other ways of increasing the product yield would be to increase promoter activity of the specific promoter regulating the expression of a specific gene of interest. Also a more general increase in the activity of several promoters at the same time could lead to an improved product yield. The control sequence may be an appropriate promoter sequence, a nucleotide sequence which is recognized by a host cell for expression of the nucleotide sequence. The promoter sequence contains transcriptional control sequences, which mediate the expression of the polypeptide. The promoter may be any nucleotide sequence which shows transcriptional activity in the host cell of choice including mutant, truncated, and hybrid promoters, and may be obtained from genes encoding extracellular or intracellular polypeptides either homologous or heterologous to the host cell.

Examples of suitable promoters for directing the transcription of the nucleic acid constructs of the present invention, especially in a bacterial host cell, are the promoters obtained from the E. coli lac operon, Streptomyces coelicolor agarase gene (dagA), Bacillus subtilis levansucrase gene (sacB), Bacillus licheniformis alpha-amylase gene (amyL), Bacillus stearothermophilus maltogenic amylase gene (amyM), Bacillus amyloliquefaciens alpha-amylase gene (amyQ), Bacillus licheniformis penicillinase gene (penP), Bacillus subtilis xylA and xylB genes, and prokaryotic beta-lactamase gene (Villa-Kamaroff et al., 1978, Proceedings of the National Academy of Sciences USA 75: 3727-3731), as well as the tac promoter (DeBoer et al., 1983, Proceedings of the National Academy of Sciences USA 80: 21-25). Further promoters are described in "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242: 74-94; and in Sambrook et al., 1989, supra.

Other useful promoters are described in WO 93/10249, WO 98/07846, and WO 99/43835 (Novozymes A/S) the contents of which are incorporated fully herein by reference. A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) are transcribed from a heterologous promoter or from an artificial promoter.

The control sequence may also be a suitable transcription terminator sequence, a sequence recognized by a host cell to terminate transcription. The terminator sequence is operably linked to the 3' terminus of the nucleotide sequence encoding the polypeptide. Any terminator which is functional in the host cell of choice may be used in the present invention.

The control sequence may also be a suitable leader sequence, a nontranslated region of an mRNA which is important for translation by the host cell. The leader sequence is operably

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linked to the 5' terminus of the nucleotide sequence encoding the polypeptide. Any leader sequence that is functional in the host cell of choice may be used in the present invention.

The control sequence may also be a polyadenylation sequence, a sequence operably linked to the 3' terminus of the nucleotide sequence and which, when transcribed, is recognized by the host cell as a signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence which is functional in the host cell of choice may be used in the present invention.

The control sequence may also be a signal peptide coding region that codes for an amino acid sequence linked to the amino terminus of a polypeptide and directs the encoded polypeptide into the cell's secretory pathway. The 5' end of the coding sequence of the nucleotide sequence may inherently contain a signal peptide coding region naturally linked in translation reading frame with the segment of the coding region which encodes the secreted polypeptide. Alternatively, the 5' end of the coding sequence may contain a signal peptide coding region which is foreign to the coding sequence. The foreign signal peptide coding region may be required where the coding sequence does not naturally contain a signal peptide coding region. Alternatively, the foreign signal peptide coding region may simply replace the natural signal peptide coding region in order to enhance secretion of the polypeptide. However, any signal peptide coding region which directs the expressed polypeptide into the secretory pathway of a host cell of choice may be used in the present invention.

Effective signal peptide coding regions for bacterial host cells are the signal peptide coding regions obtained from the genes for Bacillus NCIB 11837 maltogenic amylase, Bacillus stearothermophilus alpha-amylase, Bacillus licheniformis subtilisin, Bacillus licheniformis beta-lactamase, Bacillus stearothermophilus neutral proteases (nprT, nprS, nprM), and Bacillus subtilis prsA. Further signal peptides are described by Simonen and Palva, 1993, Microbiological Reviews 57: 109-137.

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The control sequence may also be a propeptide coding region that codes for an amino acid sequence positioned at the amino terminus of a polypeptide. The resultant polypeptide is known as a proenzyme or propolypeptide (or a zymogen in some cases). A propolypeptide is generally inactive and can be converted to a mature active polypeptide by catalytic or autocatalytic cleavage of the propeptide from the propolypeptide. The propeptide coding region may be obtained from the genes for Bacillus subtilis alkaline protease (aprE), Bacillus

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subtilis neutral protease (nprT), Saccharomyces cerevisiae alpha-factor, Rhizomucor miehei aspartic proteinase, and Myceliophthora thermophila laccase (WO 95/33836).

Where both signal peptide and propertide regions are present at the amino terminus of a polypeptide, the propertide region is positioned next to the amino terminus of a polypeptide and the signal peptide region is positioned next to the amino terminus of the propertide region.

It may also be desirable to add regulatory sequences which allow the regulation of the expression of the polypeptide relative to the growth of the host cell. Examples of regulatory systems are those which cause the expression of the gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Regulatory systems in prokaryotic systems include the lac, tac, and trp operator systems. In yeast, the ADH2 system or GAL1 system may be used. In eukaryotic systems, these include the dihydrofolate reductase gene which is amplified in the presence of methotrexate, and the metallothionein genes which are amplified with heavy metals. In these cases, the nucleotide sequence encoding the polypeptide would be operably linked with the regulatory sequence.

The present invention also relates to recombinant expression vectors comprising the nucleic acid construct of the invention. The various nucleotide and control sequences described above may be joined together to produce a recombinant expression vector which may include one or more convenient restriction sites to allow for insertion or substitution of the nucleotide sequence encoding the polypeptide at such sites. Alternatively, the nucleotide sequence of the present invention may be expressed by inserting the nucleotide sequence or a nucleic acid construct comprising the sequence into an appropriate vector for expression. In creating the expression vector, the coding sequence is located in the vector so that the coding sequence is operably linked with the appropriate control sequences for expression.

The recombinant expression vector may be any vector (e.g., a plasmid or virus) which can be conveniently subjected to recombinant DNA procedures and can bring about the expression of the nucleotide sequence. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vectors may be linear or closed circular plasmids.

The vector may be an autonomously replicating vector, i.e., a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication,

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e.g., a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome.

The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Furthermore, a single vector or plasmid or two or more vectors or plasmids which together contain the total DNA to be introduced into the genome of the host cell, or a transposon may be used.

The vectors of the present invention preferably contain one or more selectable markers which permit easy selection of transformed cells. A selectable marker is a gene the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, and the like.

Examples of bacterial selectable markers are the dal genes from Bacillus subtilis or Bacillus licheniformis, or markers which confer antibiotic resistance such as ampicillin, kanamycin, chloramphenicol or tetracycline resistance.

The vectors of the present invention preferably contain an element(s) that permits stable integration of the vector into the host cell's genome or autonomous replication of the vector in the cell independent of the genome.

For integration into the host cell genome, the vector may rely on the nucleotide sequence encoding the polypeptide or any other element of the vector for stable integration of the vector into the genome by homologous or nonhomologous recombination. Alternatively, the vector may contain additional nucleotide sequences for directing integration by homologous recombination into the genome of the host cell. The additional nucleotide sequences enable the vector to be integrated into the host cell genome at a precise location(s) in the chromosome(s). To increase the likelihood of integration at a precise location, the integrational elements should preferably contain a sufficient number of nucleotides, such as 100 to 1,500 base pairs, preferably 400 to 1,500 base pairs, and most preferably 800 to 1,500 base pairs, which are highly homologous with the corresponding target sequence to enhance the probability of homologous recombination. The integrational elements may be any sequence that is homologous with the target sequence in the genome of the host cell. Furthermore, the integrational elements may be non-encoding or encoding nucleotide sequences. On the other hand, the vector may be integrated into the genome of the host cell by non-homologous recombination.

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For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. Examples of bacterial origins of replication are the origins of replication of plasmids pBR322, pUC19, pACYC177, and pACYC184 permitting replication in E. coli, and pUB110, pE194, pTA1060, and pAMß1 permitting replication in Bacillus. The origin of replication may be one having a mutation which makes its functioning temperature-sensitive in the host cell (see, e.g., Ehrlich, 1978, Proceedings of the National Academy of Sciences USA 75: 1433).

More than one copy of a nucleotide sequence of the present invention may be inserted into the host cell to increase production of the gene product. An increase in the copy number of the nucleotide sequence can be obtained by integrating at least one additional copy of the sequence into the host cell genome or by including an amplifiable selectable marker gene with the nucleotide sequence where cells containing amplified copies of the selectable marker gene, and thereby additional copies of the nucleotide sequence, can be selected for 15 by cultivating the cells in the presence of the appropriate selectable agent.

The procedures used to ligate the elements described above to construct the recombinant expression vectors of the present invention are well known to one skilled in the art (see, e.g., Sambrook et al., 1989, supra).

The introduction of a vector into a bacterial host cell may, for instance, be effected by protoplast transformation (see, e.g., Chang and Cohen, 1979, Molecular General Genetics 168: 111-115), using competent cells (see, e.g., Young and Spizizin, 1961, Journal of Bacteriology 81: 823-829, or Dubnau and Davidoff-Abelson, 1971, Journal of Molecular Biology 56: 209-221), electroporation (see, e.g., Shigekawa and Dower, 1988, Biotechniques 6: 742-751), or conjugation (see, e.g., Koehler and Thorne, 1987, Journal of Bacteriology 169: 5771-5278).

A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) are comprised in an operon, preferably a polycistronic operon. The term "operon" in the context of the present invention means a polynucleotide comprising several genes that are clustered and perhaps even transcribed together into a polycistronic mRNA, e.g. genes coding for the enzymes of a metabolic pathway. The transcription of an operon may be initiated at a promoter region and controlled by a neighboring regulatory gene, which encodes a regulatory protein, which in turn binds to the operator sequence in the operon to respectively inhibit or enhance the transcription. The

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gene or the operon can be carried on a suitable plasmid that can be stably maintained, e.g. capable of stable autonomous replication in the host cell (the choice of plasmid will typically depend on the compatibility of the plasmid with the host cell into which the plasmid is to be introduced) or it can be carried on the chromosome of the host. The said gene may be endogenous to the host cell in which case the product of interest is a protein naturally produced by the host cell and in most cases the gene will be in it normal position on the chromosome. If the gene encoding the product of interest is an exogenous gene, the gene could either be carried on a suitable plasmid or it could be integrated on the host chromosome. In one embodiment of the invention the eubacterium is a recombinant eubacterium. Also the product of interest may in another embodiment be a recombinant protein.

The product of interest is any gene product or product of a metabolic pathway which is industrially useful and which can be produced in a bacterial cell such as a *B. licheniformis*.

In one preferred embodiment, the heterologous polypeptide(s) is an antimicrobial peptide, or a fusion peptide comprising a peptide part which in its native form has antimicrobial activity.

In another preferred embodiment, the heterologous polypeptide(s) has biosynthetic activity and produces a compound or an intermediate of interest.

Yet another embodiment relates to a host cell of the first aspect, wherein the compound or intermediate of interest comprises vitamins, amino acids, antibiotics, carbohydrates, or surfactants, and preferably the carbohydrates comprise hyaluronic acid.

In one embodiment the heterologous polypeptide(s) is an enzyme, particularly the enzyme is an enzyme of a class selected from the group of enzyme classes consisting of oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5), and ligases (EC 6). Preferably the enzyme is an enzyme with an activity selected from the group consisting of aminopeptidase, amylase, amyloglucosidase, mannanase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, galactosidase, beta-galactosidase. glucoamylase, glucose oxidase, glucosidase, haloperoxidase, hemicellulase, invertase, isomerase, laccase, ligase, lipase, lyase, mannosidase, oxidase, pectinase, peroxidase, polyphenoloxidase, phytase, phenoloxidase, protease, ribonuclease, transferase, transglutaminase, or xylanase. Preferably the enzyme is an amylase or a mannanase.

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A second aspect of the invention relates to a process for producing at least one product of interest in a *Bacillus licheniformis* mutant host cell, comprising cultivating a *B.licheniformis* mutant host cell as defined in the first aspect of the invention in a suitable medium, whereby the said product is produced. One embodiment relates to a process of the second aspect, further comprising isolating or purifying the product of interest. Suitable media for the cultivation is described below as well as methods for the purification or isolation of the produced product which is an optional additional step to the process of the present invention.

In the production methods of the present invention, the cells are cultivated in a nutrient medium suitable for production of the polypeptide using methods known in the art. For example, the cell may be cultivated by shake flask cultivation, small-scale or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or industrial fermentors performed in a suitable medium and under conditions allowing the polypeptide to be expressed and/or isolated. The cultivation takes place in a suitable nutrient medium comprising carbon and nitrogen sources and inorganic salts, using procedures known in the art. Suitable media are available from commercial suppliers or may be prepared according to published compositions (e.g., in catalogues of the American Type Culture Collection). If the polypeptide is secreted into the nutrient medium, the polypeptide can be recovered directly from the medium. If the polypeptide is not secreted, it can be recovered from cell lysates.

The medium used to culture the cells may be any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection). The media are prepared using procedures known in the art (see, e.g., references for bacteria and yeast; Bennett, J.W. and LaSure, L., editors, *More Gene Manipulations in Fungi*, Academic Press, CA, 1991).

The polypeptides may be detected using methods known in the art that are specific for the polypeptides. These detection methods may include use of specific antibodies, formation of an enzyme product, or disappearance of an enzyme substrate. For example, an enzyme assay may be used to determine the activity of the polypeptide as described herein.

The resulting polypeptide may be recovered by methods known in the art. For example, the polypeptide may be recovered from the nutrient medium by conventional procedures

including, but not limited to, centrifugation, filtration, extraction, spray-drying, evaporation, or precipitation.

The polypeptides of the present invention may be purified by a variety of procedures known in the art including, but not limited to, chromatography (e.g., ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (e.g., preparative isoelectric focusing), differential solubility (e.g., ammonium sulfate precipitation), SDS-PAGE, or extraction (see, e.g., *Protein Purification*, J.-C. Janson and Lars Ryden, editors, VCH Publishers, New York, 1989).

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A third aspect of the present invention relates to the use of a *Bacillus licheniformis* mutant host cell as defined in the first aspect for producing at least one product of interest comprising cultivating the mutant host cell in a suitable medium whereby the said product is produced, and optionally isolating or purifying the produced product.

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CLAIMS

- 1. A Bacillus licheniformis mutant host cell derived from a parent B. licheniformis host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in sporulation which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 129, wherein the mutant host cell expresses at least 5% less of the one or more polypeptide(s) involved in sporulation than the parent host cell, when they are cultivated under comparable conditions.
- The host cell according to claim 1, which is mutated by a partial or complete deletion of
 the one or more gene(s) encoding the one or more polypeptide(s) involved in sporulation.
 - 3. The host cell according to any of claims 1 2, which is mutated in two or more genes encoding two or more polypeptides involved in sporulation.
- 5. The host cell according to any of claims 1 4, which comprises one or more heterologous gene(s) encoding one or more heterologous polypeptide(s).
 - 6. The host cell according to claim 5, wherein the heterologous gene(s) is present in at least two copies.
 - 7. The host cell according to claim 5 or 6, wherein the heterologous gene(s) are stably integrated into the genome of the cell.
- 8. The host cell according to any of claims 5 7, wherein the heterologous gene(s) is integrated into the genome of the cell without leaving any antibiotic resistance marker genes at the site of integration.
 - 9. The host cell according to any of claims 5 8, wherein the heterologous gene(s) are transcribed from a heterologous promoter or from an artificial promoter.
 - 10. The host cell according to any of claim 5-9, wherein the heterologous gene(s) are comprised in an operon, preferably a polycistronic operon.
- 11. The host cell according to any of claims 5 10, wherein the heterologous polypeptide(s)
 is an antimicrobial peptide, or a fusion peptide comprising a peptide part which in its native form has antimicrobial activity.

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- 12. The host cell according to any of claims 5 10, wherein the heterologous polypeptide(s) has biosynthetic activity and produces a compound or an intermediate of interest.
- 13. The host cell according to claim 12, wherein the compound or intermediate of interest comprises vitamins, amino acids, antibiotics, carbohydrates, or surfactants.
- 14. The host cell according to claim 13, wherein the carbohydrates comprise hyaluronic acid.
- 15. The host cell according to any of claims 5 10, wherein the heterologous polypeptide(s) is an enzyme, preferably a secreted enzyme.
 - 16. The host cell according to claim 15, wherein the enzyme is is an enzyme of a class selected from the group of enzyme classes consisting of oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5), and ligases (EC 6).
 - 17. The host cell according to claim 16, wherein the enzyme is an enzyme with an activity selected from the group of enzyme activities consisting of aminopeptidase, amylase, amyloglucosidase, mannanase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, galactosidase, beta-galactosidase, glucoamylase, glucose oxidase, glucosidase, haloperoxidase, hemicellulase, invertase, isomerase, laccase, ligase, lipase, lyase, mannosidase, oxidase, pectinase, peroxidase, phytase, phenoloxidase, polyphenoloxidase, protease, ribonuclease, transferase, transglutaminase, and xylanase.
 - 18. The host cell according to claim 17, wherein the enzyme is an amylase or a mannanase.
 - 19. A process for producing at least one product of interest in a *Bacillus licheniformis* mutant host cell, comprising cultivating a *B.licheniformis* mutant host cell as defined in any of the claims 1 18 in a suitable medium, whereby the said product is produced.
 - 20. The process according to claim 19, further comprising isolating or purifying the product of interest.
- 21. A use of a Bacillus licheniformis mutant host cell as definde in any of the claims 1 18 for producing at least one product of interest comprising cultivating the mutant host cell in a suitable medium whereby the said product is produced.

22. The use according to claim 21 further comprising isolating or purifying the product of interest.

ABSTRACT

TITLE: Improved Bacillus Host Cell.

A Bacillus licheniformis mutant host cell derived from a parent B. licheniformis host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in sporulation which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 129, wherein the mutant host cell expresses at least 5% less of the one or more polypeptide(s) involved in sporulation than the parent host cell, when they are cultivated under comparable conditions.

Patent- og Varemærkestyrelsen 1 0 APR, 2002

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Lys Ile Gly Lys Thr Val Glu Ile Glu Gly Thr Tyr Asp Ile Asn Val 50 60

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tta aga gag ctt ctt Leu Arg Glu Leu Leu 55

677

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gtt cga aac tat gcg att gcg atc acg gaa acg gcg acg ccc gag ctc Val Arg Asn Tyr Ala Ile Ala Ile Thr Glu Thr Ala Thr Pro Glu Leu 95 100 105	821
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Lys Lys Ile Ser Asp Pro Gln Leu Arg Gln Leu Tyr Ser Val Ser Ala 35 40 45 Page 13

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Page 14	

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Page 15

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The Ang Cluate wie The Ang Lys Lau The Lys Lys Ser Tyr Phe Tle													
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Leu G	y Āsp His 95	Tyr Ala	Asp His	ASP Ly 100	s Ala	Leu		Tyr LO5	HIS	ASN	
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Page 22

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Ser Arg Gly Asp Met Asn Tyr His Asn His Leu Val Asn Thr Ala Asp 50 60

Thr Gly Tyr Asp Arg Pro Glu Asn Arg Lys Ile Ser Arg Asn Ile Thr 65 70 75

Gly Arg Val Asn Lys Leu Asn Tyr Val Asp Glu Ser Gln Ala Val Val 85

Thr Asn Glu Thr Val Ile Ile Ala Val Arg Ser Asp Lys Arg Leu Thr 100 105 110

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Asp Arg Thr Val Gln Val Glu Asp Asp Gly Ala Phe Thr Arg Leu 130 140

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Thr 145	Leu	аТа	Gln	Leu	A7a 150	Leu	Lys	Leu	ΑΊa	Asp 155	Gln	Аlа	Аlа	Arg	Gln 160

Lys Lys Asp Ile His Leu Glu Pro Met Pro Ser Ser Glu Arg Lys Val 165 170 175

Page 28

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Ţyŗ	Met	Leu
445		

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Arg Ala Tyr Met Ala Val Ala Gly Gly Ile Asp Val Pro Pro Val Met 115 120 125

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Page 38										

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10295.sT25.txt

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Pro Ala Gly Gly Asp Gly Gly Lys Gly Gly Asp Val Val Phe Lys Val
35 .40 43
Asp Glu Gly Leu Ser Thr Leu Met Asp Phe Arg Tyr Gln Arg His Phe 50 60
and the state of t
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Asn Ala Glu Asp Met Val Val Lys Val Pro Pro Gly Thr Val Val Ile
85 90 95
Asp Asp Asp Thr Lys Gln Val Ile Ala Asp Leu Thr Glu His Gly Gln
100 105 110
Glu Ala Val Ile Ala Lys Gly Gly Arg Gly Arg Gly Asn Thr Arg 125
Phe Ala Thr Pro Ala Asn Pro Ala Pro Gln Leu Ser Glu Asn Gly Glu 130 135 140
Dro Cly Lyc Cly And Twn Tlo Val Lou Cly Lou Lyc Val Lou Ala Asn
Pro Gly Lys Glu Arg Tyr Ile Val Leu Glu Leu Lys Val Leu Ala Asp 145 . 150 160
Val Gly Leu Val Gly Phe Pro Ser Val Gly Lys Ser Thr Leu Leu Ser
165 170 175

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val Val Ser Ser Ala Lys Pro Lys Ile Ala Asp Tyr His Phe Thr Thr 180 185 190 Leu Asn Pro Asn Leu Gly Met Val Glu Thr Glu Asp Gly Arg Ser Phe 195 200 205 Val Met Ala Asp Leu Pro Gly Leu Ile Glu Gly Ala His Glu Gly Val 210 215 220 Gly Leu Gly His Gln Phe Leu Arg His Ile Glu Arg Thr Arg Val Ile 225 230 235 240 Val His Val Ile Asp Met Ser Gly Leu Glu Gly Arg Asp Pro Tyr Glu 245 250 255 Asp Tyr Val Thr Ile Asn Lys Glu Leu Glu Gln Tyr Asn Leu Arg Leu 260 265 270 Thr Glu Arg Pro Gln Ile Ile Val Ala Asn Lys Met Asp Met Pro Asp 275 280 285 Ala Glu Glu Asn Leu Lys Ala Phe Lys Glu Lys Leu Thr Asp Asp Tyr 290 295 300 Pro Val Phe Pro Ile Ser Ala Val Thr Arg Gln Gly Leu Arg Asp Leu 305 310 315 320 Leu Phe Glu Ile Ala Asp Arg Leu Glu Thr Thr Pro Glu Phe Pro Leu 325 330 335 Tyr Asp Glu Glu Asp Met Ala Glu Asn Arg Val Met Tyr Lys Leu Glu 340 345 Asp Glu Glu Ala Pro Phe Glu Ile Ser Arg Asp Pro Asp Gly Thr Phe 355 360 365 Val Leu Ser Gly Ala Lys Leu Glu Arg Leu Phe Lys Met Thr Asp Phe 370 380 Ser Arg Asp Glu Ser Val Lys Arg Phe Ala Arg Gln Leu Arg Gly Met 385 390 395 400 Gly Val Asp Asp Ala Leu Arg Ala Arg Gly Ala Lys Asp Gly Asp Thr 405 410 415 Ile Arg Leu Leu Glu Phe Glu Phe Glu Phe Ile Asp 420 425

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Arg Gly Lys Tyr Ile Lys Ala Ile Lys Ala Tyr Arg Ala Ala Glu Lys 110 115	
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Page 42	

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Glu Ile Glu Gly Gln Gly Arg Lys Leu Ser Gly Leu Leu Glu Tyr Tyr 85 90 95

Phe Ser Phe Phe Thr Gly Met Tyr His Phe Ser Arg Gly Lys Tyr Ile 100 105 110

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att gaa aag atg gaa gaa gat cat gac gtt ctt ctc tat tat caa at Ile Glu Lys Met Glu Glu Asp His Asp Val Leu Leu Tyr Tyr Gln Me 30 35 40	g 629 t
ctg gat ttt cgc tta agg ctt ctt ctt gaa gat atc tcc caa tct tc Leu Asp Phe Arg Leu Arg Leu Leu Glu Asp Ile Ser Gln Ser Se 45 50 55	c 677 r
aca gaa aaa ttg gaa gcc atc agt ttt aag gac aaa gat cca aaa ag Thr Glu Lys Leu Glu Ala Ile Ser Phe Lys Asp Lys Asp Pro Lys Se 60 65 70 75	r
acg gac gat aag ctg aat tat tat ttt tat ctg ttc aaa ggg att ta Thr Asp Asp Lys Leu Asn Tyr Tyr Phe Tyr Leu Phe Lys Gly Ile Ty 80 85	it 773
gaa gac tac aag caa aac cat aca gaa gcg ctt aat ttt ttc aga at Glu Asp Tyr Lys Gln Asn His Thr Glu Ala Leu Asn Phe Phe Arg Il 95 100	a 821 e
gcg gaa aaa agg ctg agc gtc att caa aat gaa att gaa aaa gcc ga Ala Glu Lys Arg Leu Ser Val Ile Gln Asn Glu Ile Glu Lys Ala Gl 110 115 120	aa 869 Iu
ttt cat tat aaa atc ggt gtt ttg tat tac aac tta aaa gcg aca tg Phe His Tyr Lys Ile Gly Val Leu Tyr Tyr Asn Leu Lys Ala Thr Tr 125 130 135	gg 917 °p
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Page 46

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Asp Phe Ile His Gln Leu Glu Asp Lys Lys Ala Trp Val Asp Leu Glu 305 310 315 320

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Page 51

Leu Ile Tyr Tyr Ser Leu Leu Glu Leu Arg His Lys Ile Met Leu Tyr 50 60 Asp Thr Arg Gly Lys Lys Ile Glu Gln Gln Glu Glu Leu Thr Asn Gly 65 70 75 80 Gly Ser Ala Ala Ser His Met Thr Ser Tyr Tyr Tyr Leu Phe Ser Gly Ala Tyr Glu Val Tyr Lys Lys Asn Tyr Glu Gln Ala Ile Ser Phe 100 105 110 Tyr Lys Ile Ala Glu Lys Lys Leu Ala His Val His Asp Glu Ile Glu 115 120 125 val Ala Gln Phe His Asp Lys Val Gly Lys Leu Tyr Tyr Leu Gly 130 140 Gln Asn Ile Val Ser Leu Asn His Thr Arg Gln Ala Met Glu Ile Phe 145 150 155 160 Lys Gly His Gly Asp His Asp Met Asn Leu Val Ser Thr Tyr Ile Thr 165 170 175 Met Ala Gly Asn Tyr Thr Glu Met Gly Lys Tyr Thr Glu Ala Glu Glu 180 185 190 Tyr Leu Thr Glu Ala Ile His Thr Val Arg Lys Ala Gly Asp Cys Phe 195 200 205 Lys Glu Met Gln Leu Leu His Asn Phe Ala Leu Leu Tyr Ala Ala Met 210 220 Asp Asn Ser Glu Lys Ser Ile Gln Phe Leu Glu Ile Val Leu Asp Asp 225 230 235 Gln Ala Tyr Ala Ala Ser Asp Tyr Tyr Phe Asn Ala Val Phe Leu Met 245 250 255 Ile Lys Glu Leu Phe Lys Val Gly Asp His Lys Arg Ala Ala Ala Phe 260 265 270 Tyr Lys Glu Gly Lys Glu Arg Ser Lys Ser Ala Ala Asn Lys Ile Phe 275 280 285 Asp Ala Lys Ile Asp Ile Leu Tyr Ala Ala Tyr Ala Gly Asp Gly Glu 290 295 300 Gln Ala Val Lys Asp Cys Lys Asp Asn Ile Glu Ile Leu Phe Gln Thr 305 310 315 320 Page 52

Lys Gln Tyr Asp Ser Ala Arg Glu Leu Ser Leu Leu Thr Ala Asn Val 325 330 335

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Ser Leu Glu Lys Ile Ala Glu Arg Tyr Glu Val Asp Phe Glu Glu Leu 15 20 25	
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Lys Lys Leu Asn Ser Gln Leu Ser Asn Pro Asp Leu Ile Met Pro Gly 30 35 40	
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7)	605
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<213> Bacillus licheniformis

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Ser Gly Gly Val Pro Val Lys Lys Glu Glu Gln Leu Asn Met Arg Lys 50 60

Glu Leu Pro Lys Lys Gln Gln Glu His Pro Phe Ala Lys Glu Lys Pro 80

Lys Ser Lys Leu Asp Val Glu Asp Ile Lys Pro Lys Glu Lys Pro Ser 85 90 95

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Glu Gly Asp Ile Ser Asn Leu Tyr Gln Ser Val Asn Gln Leu His Gln 115 120 125

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tcg tca aa Ser Ser Ly	a ccg tcc (s Pro Ser / 80	gca gaa tac Ala Glu Tyr	gcg atc o Ala Ile I 85	ccg ttt gca Pro Phe Ala	aca ggg Thr Gly 90	tgt 773 Cys
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tcc tgc ac ser Cys Th 140	a tcc gat r Ser Asp	atc gtc gga Ile Val Gly 145	att gac	cat ttg aca His Leu Thr 150	cat acg His Thr	tta 965 Leu 155
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Ala Leu Pro Gln His Ala Arg Arg Asp Ile Thr Phe Glu Met Ile Gln 260 265 270

His Arg Phe Thr Lys Pro Ala Lys Arg Val Ile Glu Lys Asn Tyr Pro 275 280 285

Lys Thr Lys Leu Glu Leu Asp Glu Glu Lys Arg Arg Tyr Lys Trp Gly 290 295 300

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ttt green per service property of the per service property	tt gat al Asp 95 tg gtt et Val ga ggt	cag Gln gct Ala gac Asp agc 245 gga gga Gly	ttc Phe ggc Gly 230 acg Thr	aaa Lys cgc Arg 215 gga Gly ctg Leu	ata Ile 200 Cgc Arg aaa Lys atg	tac Tyr gaa Glu ggc Gly gat Asp	gta Val aaa Lys gga Gly ttt Phe 250	aaa Lys tat Tyr gac Asp 235 aga Arg	ggc Gly gtg Val 220 gtc Val tat	gga Gly 205 cca Pro gtt val caa Gln	gac Asp aaa Lys ttc Phe aga Arg	g ac gga gga ggy aaa Lys cats 255 ggc	ggc Gly ggc Gly gtt Val 240 ttt Phe	aac Asn CCt Pro 225 gac Asp aag Lys	1162 1210 1258
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Met Asn Lys Leu Gln Leu Ile Lys Gly Asn Leu Thr Leu L 35 40 45	ys Lys.	туг												
Asp Arg Val Phe Glu Ile Ile Asp Glu Val Val Ile Glu A	Ala Gln	His												
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Ser Asp Asn His Leu Thr Ile Thr Leu Gln Thr Asp Gly 130 135 140	Pro Asp	Asp												
Arg Leu Val Ile Phe Leu Asp Phe His Gly Val Phe Thr 1 145 150 155	Lys Leu	Thr 160												
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Asp Glu Gly Leu Ser Thr Leu Met Asp Phe Arg Tyr Gln Arg His Phe 50 60

Lys Ala Ala Arg Gly Glu His Gly Met Ser Lys Asn Gln His Gly Arg 65 70 75

Asn Ala Glu Asp Met Val Val Lys Val Pro Pro Gly Thr Val Val Ile 85 90 95

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Bacillus licheniformis

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Page 69

917

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ccg Pro	cti Let 285	ı Sei	c gct r Ala	gac A Asp	ggo Gly	gaa / Glu 290	i bud	t gad e Asp	gco Ala	ato a Ilo	gte Va 29		t cc	g aaa D Lys	a atg s Met	1397
Lei	g gco u Ala	gga	a aad y Asi	c cco	g gto Va 30	i Ly:	a cae s Hi:	c gt s Va	t tci I Sei	t taa	aact	tgaa	gtc	tgtta	aca	1447
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1867 1927 1928

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Leu Le	u Leu	Leu 20	Met	Met	Thr	аlа	Phe 25	Ile	Leu	Lys	Arg	Arg 30	val	Lys
Lys Ar	g Arg 35	Leu	Ile	Leu	GТу	д1а 40	Phe	val	Аlа	Ser	ser 45	Ile	val	Leu
Phe Me 50		Thr	Pro	Phe	Ser 55	Pro	туг	۷a٦	Leu	ніs 60	Pro	Аlа	G ¶y	Lys
Leu Se 65	r Phe	Ser	٧a٦	va1 70	Ile	val	Leu	val	д]а 75	Phe	GТу	Phe	Lys	Arg 80
Phe Ar	g Phe	Phe	Leu 85	Gln	Asn	Leu	Phe	Ser 90	Phe	туг	Phe	Ala	т h r 95	Phe
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Asp Pr 13	o Val	ser	Trp	Leu	Phe 135	٧a٦	Cys	٧a٦	ςΊу	Phe 140	Ala	Αla	v a1	Trp
Leu Ph 145	ie Ser	Lys	Lys	Arg 150	Phe	Glu	Asp	· Ala	G]u 155	- Ala	Lys	Lys	.Ile	G]n 160
Tyr G	lu Glu	Arg	Va1 165	Arg	Leu	Glu	ı Ala	Cys 170	Ile	Gly	Glu	His	Thr 175	Leu
His Pł	ne Thr	Gly 180	Leu	IJе	Asp	Ser	G]y	Asn	Gln	Leu	Tyr	Asp 190	Pro	Ile

Thr Lys Thr Pro Val Met Ile Val Asn Ile Glu Lys Leu Lys Val Val 195 200 205 Leu Gly Glu Glu Ala Ser Val Thr Ile Lys Glu Met Ser Pro Leu Asp 210 220 Ala Val Gly Lys Leu Asp Glu Ala Leu Pro Tyr Ile Gly Arg Ile Arg 225 230 235 Leu Ile Pro Tyr Arg Gly Val Gly His Gln His Gln Phe Leu Leu Cys 245 250 255 Leu Lys Pro Asp His Val Leu Val Cys Thr Glu Arg Glu Val Ile Glu 260 265 270 Ala Pro Lys Cys Leu Ile Gly Ile Ser Thr Ser Pro Leu Ser Ala Asp 275 280 285 Gly Glu Phe Asp Ala Ile Val His Pro Lys Met Leu Ala Gly Asn Pro 290 295 300 val Lys His Val Ser <210> 52 1922 <211> <212> DNA Bacillus licheniformis <213> <220> <221> **CDS** (501)..(1421) <222> <223>

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ctg Leu 140	att Ile	atc Ile	ggt Gly	CCG Pro	ccg Pro 145	caa Gln	acc Thr	gga Gly	aaa Lys	aca Thr 150	aca Thr	ctg Leu	ctc Leu	aga Arg	gac Asp 155	· 965
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cca Pro	aaa Lys 205	Ala	gaa Glu	899	ctg Leu	atg Met 210	atg Met	atg Met	atc Ile	aga Arg	tcg ser 215	Met	agt Ser	ccg Pro	gag Glu	1157
gta Val 220	atg Met	atc Ile	gcc Ala	gat Asp	gag Glu 225	Ile	ggg	aga Arg	atg Met	gaa Glu 230	ASP	gca Ala	gaa Glu	gcg Ala	ctc Leu 235	1205
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Val Val Glu Asn Gly Ala Val Lys Gly Ile Arg Glu Ile Ser Ser Phe 100 Page 81

Asn Ile Arg Ile Ala Lys Glu Lys Ile Gly Ile Ser Lys Pro Tyr Val 115 120 125 Pro His Leu Phe Gln Asn Ser Trp Leu Asn Thr Leu Ile Ile Gly Pro 130 140 Pro Gln Thr Gly Lys Thr Thr Leu Leu Arg Asp Leu Ala Arg Leu Ile 145 150 160 Ser Ser Gly Ser Gly Asn Ala Pro Ala Lys Lys Val Gly Ile Val Asp 170 175 Glu Arg Ser Glu Ile Ala Gly Cys Val Asn Gly Ile Pro Gln Tyr Arg 180 185 190 Leu Gly Asp Arg Ala Asp Ile Leu Asp Ala Cys Pro Lys Ala Glu Gly 195 200 205 Leu Met Met Met Ile Arg Ser Met Ser Pro Glu Val Met Ile Ala Asp 210 220 Glu Ile Gly Arg Met Glu Asp Ala Glu Ala Leu Leu Glu Ala Val His 225 230 235 240 Ala Gly Val Thr Val Ile Val Ser Ala His Gly Tyr Thr Tyr Ala Asp 245 250 255 Leu Ala Arg Arg Pro Ser Leu Lys Met Leu Gln Glu His Arg Val Phe 260 265 270 Glu Arg Ile Val Glu Leu Ser Arg Lys Asn Gly Pro Gly Ser Leu Ser 275 280 285 Arg Ile Leu Asn Gly Asn Gly Glu Pro Leu Gly Ala Ala Lys Arg Met 290 295 300 Leu Ser Cys 305

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	ggtga C												360
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	.actya a cggtc C												480
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aca (jca gcc	aca too	- . aca (nga ttt	gaa	ata	aca	aaq	cct	ttc	agg	g <u>a</u> a	578
Ala A	la Ala	Thr Tri	Thr	STy Phe	Glu 20	Met	ĂΊã	Lyš	Pro	Phe 25	Arg	Glu	
200 (cg aag	caa ato	- כמכ ו	caa cta	tta	acc	act	ttg	cag	tct	ttg	gag	626
Arg I	ro Lys	GIn II	e Arg	Gln Leu 35	Leu	Ăla	ĂΊα	Leŭ	G] n 40	Ser	Leu	Glu	
act (*** ***	ato ta	- 000	cat aca	cca	ctc	cat	cag	gca	tca	aaa	cag	674
Ala (Slu Ile 45	Met Ty	GIY	His Thr 50	-Pro	Leu	Arg-	G]ñ 55	Āla	ser	Lys	Gin	
245	762 636	cad ct	r acc	מאט ככט -	ata	qcc	tct	ttg	ttt	cag	aça	tţt	722
ile 7	Ala His	Gin Le	Thr 65	Glu Pro	٧a٦	Ăla	Ser 70	Leu	Phe	Gln	Thr	Phe 75	
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Ala	Glu Gln	Leu Gl	u Lys	Gly Ser	ĂĨa	Ser 85	ĂΊa	ĞĨÿ	Thr	Āla	Trp 90	Glu	
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guc	~3~ ~~3	3-3 -4	9	-99 -30	J	Pag	je 83	3		_			

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Gly His Thr Pro Leu Arg Gln Ala Ser Lys Gln Ile Ala His Gln Leu 50 60

Thr Glu Pro Val Ala Ser Leu Phe Gln Thr Phe Ala Glu Gln Leu Glu 65 70 75 80

Lys Gly Ser Ala Ser Ala Gly Thr Ala Trp Glu Asp Ser Leu Glu Lys 90 95

Val Trp Pro Glu Thr Ala Leu Lys Lys Glu Tyr Glu Ile Leu Arg 100 105 110

Gln Phe Gly Glu Thr Leu Gly Arg His Asp Leu Ile Ser Gln Gln Lys 115 120 125

His Ile Lys Leu Ala Leu Thr His Leu Glu Thr Glu Glu Ala Glu Ala 130 135 140

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cagaaaggag gatttcctga gtg aag cgt ttt ctg ttc tgg ctc ttg gtc atc Val Lys Arg Phe Leu Phe Trp Leu Leu Val Ile 10	-
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gcg Ala	gag Glu	ccg Pro 30	gct Ala	ggg Gly	gaa Glu	acc Thr	gct Ala 35	gca Ala	gaa Glu	gaa Glu	tcg Ser	gca Ala 40	gaa Glu	gcc Ala	att Ile	629
gca Ala	aga Arg 45	gag Glu	cag Gln	gct Ala	gaa Glu	ggt Gly 50	ttg Leu	gaa Glu	cta Leu	gac Asp	cgg Arg 55	gtc Val	ggg Gly	gag Glu	ttc Phe	677
tgg Trp 60	aac Asn	aac Asn	att Ile	ttg Leu	aca Thr 65	gag Glu	tat Tyr	ggg Gly	gga Gly	cac His 70	ctt Leu	ccc Pro	gaa Glu	agt Ser	caa Gln 75	725
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gag Glu	gaa Glu	tgg Trp	ggc Gly 95	aaa Lys	gcg Ala	ctg Leu	ttt Phe	tcc Ser 100	tac Tyr	ttg Leu	ttc Phe	cat His	gaa Glu 105	gtg Val	ctg Leu	821
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gtc Val	ctg Leu 125	Leu	cag Gln	ctt Leu	ttg Leu	caa Gln 130	ASI	gcg Ala	ttt Phe	caa Gln	caa Gln 135	ago Ser	acc Thr	gtc Val	agc Ser	917
aaa Lys 140	: val	gcg Ala	tat Tyr	gca Ala	att Ile 145	vai	tac Tyr	atg Met	gtg Val	ctg Leu 150	110	att Ile	ctt Leu	gcg	ctc Leu 155	965
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atç Me1	aca Thr	ago Ser	ttt Phe 175	: Ile	ctg Leu	tcg Ser	cto Leu	gta Val 180	Pro	ctg Leu	ctt Leu	cto Lei	g gcg i Ala 185		atg Met	1061
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ct1 Le1	t tt1 J Phe 20:	e Lei	ato u Mei	g aad t Asr	acg Thr	ago Ser 210	. G13	ttg Lei	j ttt i Phe	ato Elle	caa Glr 215	ַעיייַ	t ato	gte Va	ttg Leu	1157
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gc AT	a aa a Ly 28	5 Ph	c at e Il	t ac e Th	c gg r Gl	a aa y As 29	Ņ PN	c at e Il	e Pr	c gt o Va	29	<u>u</u> G	c cg y Ar	c at g Me	g ttt t Phe	1397

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	acc Thr	gtc Val	ggg Gly	ata Ile	ctc Leu 320	ggt Gly	gtg Val	gca Ala	atc Ile	tta Leu 325	att Ile	tgc Cys	atc Ile	gca Ala		ttt Phe	1493
		gcg Ala		aaa Lys 335	atc Ile	ctt Leu	tcc Ser	ctc Leu	gcg Ala 340	ctc Leu	ata Ile	tac Tyr	aaa Lys	att Ile 345	gcc Ala	gcg Ala	1541
	gcg Ala	gtt Val	ctc Leu 350	cag Gln	cct Pro	ctc Leu	gga Gly	ggc Gly 355	ggc Gly	ccg Pro	gtt Val	atc Ile	agc Ser 360	tgc Cys	ctg Leu	gat Asp	1589
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Leu Ser Leu Ala Leu Ile Tyr Lys Ile Ala Ala Ala Val Leu Gln Pro 340 345	
Leu Gly Gly Gly Pro Val Ile Ser Cys Leu Asp Val Ile Ser Lys Ser 365	
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ggc cac ctg ctc ccg aaa gac gag aaa gac gga aaa aag ctg acg aaa Gly His Leu Leu Pro Lys Asp Glu Lys Asp Gly Lys Lys Leu Thr Lys 15 20 25	581

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cag Gln 60	ccg Pro	gcg Ala	gct Ala	tca Ser	caa G1n 65	aaa Lys	gct Ala	acg Thr	tct Ser	gaa Glu 70	agc Ser	acc Thr	gta Val	cag Gln	agc Ser 75	725
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gto Va 220	gci I Ala	t gti a Va	t gco I Ala	cct Pro	aaa Lys 225	aaa Lys	atg Mei	aag Lys	gag Gli	g gat i Asp 230	t tca 5 Sei	a taa	aatga	atgc		1203
													tgta	ctga	agtgtct	1263
															caagttg	
										-					gataccg	
															gcgagtg	
															acaacat	
															atcgtca	
															gagctca	
gc	gaag	ctga	agg	aacg	gaa a	aaga	ccct	tg a	aacc Pa	ctca age S	t ca 93	aaac	aaaa	ggc	tattaag	1683

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Ser Ser Glu Pro Ser Gln Glu Pro Ala Ala Asp Gln Pro Ala Ala Ser 50 60

Gln Lys Ala Thr Ser Glu Ser Thr Val Gln Ser Gly Glu Gly Glu Lys 75 70 80

Glu Val Phe Lys Pro Ala Ser Asp Asp Lys Pro Lys Glu Ser Ile Gln 85 90 95

Asp Tyr Glu Gln Glu Tyr Glu Asn Gln Leu Lys Asp Ile Leu Glu Thr 100 105

Ile Ile Gly Val Glu Asp Val Ser Ile Val Val Asp Val Asp Ala Thr 115 120 125

Ser Leu Lys Ile Phe Glu Lys Asn Arg Lys Thr Gln Glu Thr Ser Thr 130 135 140

Asn Glu Thr Asp Lys Gln Gly Gly Lys Arg Thr Val Ser Glu Met Ser 145 150 160

Ser Asp Glu Glu Ile Val Ile Ile Lys Asp Gly Asp Lys Glu Thr Pro 165 170 175

Val Val Gln Thr Lys Lys Pro Asp Ile Arg Gly Val Leu Val Val 180 185 190

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acc atg tta agt ctc gtc gtt gta ctg agt gtc tac tac att atg tcg Thr Met Leu Ser Leu Val Val Val Leu Ser Val Tyr Tyr Ile Met Ser 15 20 25	578
ccc gaa gga gaa aat gtc gtc acg gtt gat gac aag gaa caa gtt gcc Pro Glu Gly Glu Asn Val Val Thr Val Asp Asp Lys Glu Gln Val Ala 30 35 40	626
gct gaa aaa gaa aaa ccg atg aaa gaa gag cct gcc aag gat ggc aaa Ala Glu Lys Glu Lys Pro Met Lys Glu Glu Pro Ala Lys Asp Gly Lys 45 50 55	674
gat gat acc gcg cct gct aaa gac aaa act aaa ggg aaa gat aca aaa Asp Asp Thr Ala Pro Ala Lys Asp Lys Thr Lys Gly Lys Asp Thr Lys 60 75	722
gat aaa gaa acg tct gcg agt gag cag aac gga gag gtt gtc aca gag Asp Lys Glu Thr Ser Ala Ser Glu Gln Asn Gly Glu Val Val Thr Glu 80 85 Page 95	770

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Pro Met Lys Glu Glu Pro Ala Lys Asp Gly Lys Asp Asp Thr Ala 50 60	Pro
Ala Lys Asp Lys Thr Lys Gly Lys Asp Thr Lys Asp Lys Glu Thr 65	ser 80
Ala Ser Glu Gln Asn Gly Glu Val Val Thr Glu Glu Ser Ser Gly	Asp
85 90 93	
Glu Asp Leu Phe Thr Thr Tyr Arg Met Glu Met Asp Asp Gln Arg	

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Tyr

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tta atc tgt atc gcc atc tcg att att gcg gtt ttg cag ctt ggc gta Leu Ile Cys Ile Ala Ile Ser Ile Ile Ala Val Leu Gln Leu Gly Val 30 35 40	629
gca ggg caa acg ttc att tac atg ttc cgc ttt ttc gcc ggt gaa tgg Ala Gly Gln Thr Phe Ile Tyr Met Phe Arg Phe Phe Ala Gly Glu Trp 45 50 55	677
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Page 97	

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aag Lys 220	Lys	tcc Ser	gga - G1y	aaa Lys	aaa Lys 225	GIN	aag Lys	acg Thr	cag Gln	aga Arg 230	g Ly.	a cc	g aaa o Ly:	a gt s Va	g	tct Ser 235	1205
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tca Ser	gaa Glu	a cco i Pro	att 5 Ilo 25	e Ile	t tca e Ser	a ago Ser	tt1 Phe	t tco e ser 260	. wat	cg Arg	t ga g Ası	t ga p G1	a aa u Ly 26		ro	gaa Glu	1301
gt <u>i</u> Va	t car	g gc1 n Ala 270	a ıyı	c gaa r Glu	a gct u Ala	t cco	g gcg 5 A1: 27:	2 710	t cct a Pro	t gc	t ga a Gl	a cc u Pr 28	•	t g o A	ct la	gag Glu	1349
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ta Ty	c ga r As	c aa p As	t gc n Al 33	a Ar	g aa g Ly	g ct s Le	g ga u Gl	a ag u Ar 34	9 '''	g tt r Ph		a ag n Se	21 F1	ic g ie G 15	ga ily	gtt Val	1541

Page 98

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Ser Asp Asp Leu Ala Leu Ala Ala Lys Asp Ile Arg Ile Glu	
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gca gga gcg acc gga agc ggg aaa agc gtc tgt gtc aac ggg atc att 19 Ala Gly Ala Thr Gly Ser Gly Lys Ser Val Cys Val Asn Gly Ile Ile 460 465 470	925
aca agc att ttg atg agg gca aag ccc cac gaa gtg aag atg atg atg Thr Ser Ile Leu Met Arg Ala Lys Pro His Glu Val Lys Met Met 480 480 485	973
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aag aaa gtc gtc aac gaa atg gag cgg cgc tac gaa ttg ttt tct cac 2 Lys Lys Val Val Asn Glu Met Glu Arg Arg Tyr Glu Leu Phe Ser His 525 530 535	117
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Page 99

gtc Val 620	att Ile	aaa Lys	gcc Ala	aac Asn	att Ile 625	ccg Pro	tca Ser	agg Arg	atc Ile	gct Ala 630	ttc Phe	agc Ser	gta Val	tcg Ser	tct Ser 635	2405
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Ser Ser Phe Ser Asp Arg Asp Glu Lys Pro Glu Val Gln Ala Tyr Glu 260 265 270 Ala Pro Ala Ala Pro Ala Glu Pro Pro Ala Glu Pro Glu Ile Gly Glu 275 280 285 Glu Met Gln Ala Ser Gly Ala Pro Glu Ile Thr Phe Thr Glu Leu Glu 290 295 300 Asn Lys Asp Tyr Gln Leu Pro Ser Ile Gln Leu Leu Asp Asp Pro Lys 305 310 315 His Thr Gly Gln Gln Ala Asp Lys Lys Asn Ile Tyr Asp Asn Ala Arg 325 330 335 Lys Leu Glu Arg Thr Phe Gln Ser Phe Gly Val Lys Ala Lys Val Thr 340 350 Gln Val His Leu Gly Pro Ala Val Thr Lys Tyr Glu Val Tyr Pro Asp 355 360 365 Val Gly Val Lys Val Ser Lys Ile Val Asn Leu Ser Asp Asp Leu Ala 370 375 Leu Ala Leu Ala Ala Lys Asp Ile Arg Ile Glu Ala Pro Ile Pro Gly 385 390 395 400 Lys Ser Ala Ile Gly Ile Glu Val Pro Asn Ala Glu Val Ala Met Val 405 410 415 Ser Leu Lys Glu Val Leu Glu Ser Lys Leu Asn Asp Arg Pro Asp Ala 420 430 Lys Leu Met Ile Gly Leu Gly Arg Asn Ile Ser Gly Glu Ala Val Leu 435 445 Ala Glu Leu Asn Lys Met Pro His Leu Leu Val Ala Gly Ala Thr Gly
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Glu Met Glu Arg Arg Tyr Glu Leu Phe Ser His Thr Gly Thr Arg Asn 530 540 Ile Glu Gly Tyr Asn Asp Tyr Ile Lys Arg Met Asn Ala Ala Glu Glu 545 550 560 Ala Lys Gln Pro Glu Leu Pro Tyr Ile Ile Val Ile Val Asp Glu Leu 565 570 Ala Asp Leu Met Met Val Ala Ser Ser Asp Val Glu Asp Ser Ile Thr 580 585 590 Arg Leu Ser Gln Met Ala Arg Ala Ala Gly Ile His Leu Ile Ile Ala 595 600 605 Thr Gln Arg Pro Ser Val Asp Val Ile Thr Gly Val Ile Lys Ala Asn 610 620 Ile Pro Ser Arg Ile Ala Phe Ser Val Ser Ser Gln Thr Asp Ser Arg 625 630 640 Thr Ile Leu Asp Met Gly Gly Ala Glu Lys Leu Leu Gly Arg Gly Asp 645 650 Met Leu Phe Leu Pro Val Gly Ala Asn Lys Pro Leu Arg Val Gln Gly 660 670 Ala Phe Leu Ser Asp Glu Glu Val Glu Lys Val Val Asp His Val Ile 675 680 685 Ser Gln Gln Lys Ala Gln Tyr Gln Glu Glu Met Ile Pro Glu Glu Thr 690 695 700 Gln Glu Thr Val Ser Glu Val Thr Asp Asp Leu Tyr Asp Glu Ala Val 705 710 720 Ala Leu Val Val Ser Met Gln Thr Ala Ser Val Ser Met Leu Gln Arg 725 730 735 Arg Phe Arg Ile Gly Tyr Thr Arg Ala Ala Arg Leu Ile Asp Ala Met 740 750 Glu Glu Arg Gly Ile Val Gly Pro Tyr Glu Gly Ser Lys Pro Arg Glu 755 760 765 Val Leu Leu Ser Lys Glu Gln Tyr Glu Glu Leu Ser Ser 770 780

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Αla	Gly	Thr	· Glu	Ser 85	· Ala	Leu	Ser	· Ala	Phe 90	Pro) Pro	Arg	, Pro	6]u 95	ı А]а	
GΊχ	/ Asp	Glr	His 100		Phe	G Tu	ı Arg	Ser 105	•	Lys		s Ser	Arg 110	Thr	· Gly	

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10295.5T25.txt

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Thr Glu Lys Glu Asn Arg Arg Gly Lys Thr Ser Ser Thr Ile Asp Gly 175	
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Page 108	

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Lys Pro Val Ser Ser Ile Glu Lys Ala Met Glu Glu Gln Ala Ser Glu 225 230 235 240

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val	Lys	Glu	Pro	Ala 245	Gln	Pro	ser	۷a٦	G]u 250	Glu	Lys	ser	Lys	Thr 255	Glu
Asp	Lys	Αla	Lys 260	Asp	Gln	Thr	Asp	G]y 265	Lys	Asp	Asp	Lys	Thr 270	Lys	Arg
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<211> 248

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Leu His Tyr Leu Ile Val Ala Val Phe Phe Thr Leu Thr Asp Ala Phe 65 70 75

Ile Phe Leu Asn Ala Tyr Phe Lys Lys Leu Gly Ser Ser Glu Leu Ala 85 90 95

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Tyr Gln Asn Arg Leu Lys Thr Phe Gln Tyr Leu Leu Lys Asn Glu Pro 115 120 125

Ile His Ile Tyr Tyr Gly Asn Ile Glu Ala Tyr Ala Glu Gly Ile Glu 130 135 140

Lys Leu Ile Lys Arg Phe Ala Glu Lys Met Asn Ile Ser Ala Ala Leu 145 150 160

Cys Glu Tyr Asn Ser Glu Glu Ser Lys Asp His Leu Leu Glu His Met 165 170 175

Glu Asn Arg Phe Asp Val Gln Glu Lys Leu Asp Arg Lys Asp Val Tyr 180 185 190

Tyr Glu Glu Asn Gly Lys Met Val Leu Ile Pro Phe Ser Ile His Asp 195 200 205

Phe Asp Tyr Val Met Lys Leu Thr Ser Glu Asp Leu Val Thr Glu Phe 210 215 220 Page 113

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Caa tta caa act tta cgt caa tat act cag tta tat ggt tgg gag att Gln-Leu-Gln-Thr-Leu Arg Gln Tyr Thr Gln Leu Tyr Gly Trp Glu Ile 30 35 40	629
gca gag gaa tat gta gat gag gga ata agt gga aag aac att agc ggt Ala Glu Glu Tyr Val Asp Glu Gly Ile Ser Gly Lys Asn Ile Ser Gly 45 50 55	677
Cgc cct gca atg caa aaa ctt att tca gat gtt gaa aag gat aaa ttt Arg Pro Ala Met Gln Lys Leu Ile Ser Asp Val Glu Lys Asp Lys Phe 65 70 75	725
Caa gct gtt ctt gtt tgg aag atc tca cgc cta tca cga aat atg tta Page 114	773

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Gln	Ala	Va T	Leu	80	тгр	Lys	Tie	ser	85 85	Leu	261	Aig	ASII	90	Leu	
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10295.ST25.txt
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Arg Ile Val Thr Trp Lys His Leu Ser Lys Arg Pro Tyr Gln 405

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Page 119

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115 120 125 Asp Pro Leu Ala Phe Phe Gln Pro Lys Gly Ala Lys Pro Ser Gly Gln 130 140 Val Glu Val Asn Gln Asp Leu Ala Val Pro Ala Val Gly Lys Val Gln 145 150 160 Glu Lys Phe Ser Gly Gln Gly Ile Lys Val Glu Thr Glu Asp Glu Thr 165 170 175 Ile Arg Ser Met Lys Glu Gly Tyr Val Ile Glu Val Asp Lys Asn Pro 180 185 Glu Thr Gly Leu Thr Val Val Leu Gln His Ala Asp Asn Ser Tyr Thr 195 200 205 Tyr Tyr Gly Gln Leu Lys Lys Ala Asp Val Ala Leu Tyr Asp Tyr Ile Asp Lys Gly Thr Lys Leu Gly Thr Ile Glu Gln Asp Lys Asn Gln Lys 225 230 240 Gly Ile Tyr Tyr Phe Ala Ile Lys Gln Gly Glu Glu Phe Val Asp Pro 245 250 255

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Val Leu Gly Val Trp Thr Asn Met Phe Lys Leu Ala Gly Asn Val Ile 115 120 125										
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1 5 10 15	
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										T02	95	. 51	23.	LXL							
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99 61	у H [.]	at is	gto Val	ate 110 25	e va	g a	tc g le \	gtc /al	gat Asp	acg Thi 260		cg er	cca Pro	ag Se	c g r V	tc al	atc Ile 265	at Il	e e	aca Thr	1301
cc Pr	g a o T	cc hr	act Thi	· Le	g tt u Ph	t c ne H	ac d is I	cat His	gtt Val 275	ca Gli	g C	at is	gct Ala	ga G1	g g u G	aa Tu 80	tac Tyr	ag Ai	ga rg	cag Gln	1349
ac Th	ır P	cg ro 85			t gg	gg a ly T	111	ttt Phe 290	tta Leu	ag Ar	g t g T	gg rp	gtg Val	cg Ar 29		tt he	ttc Phe	g	gt ly	att Ile	1397
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g A	la	atg Me1 36!	g gg		tg a	itc [le	gcc Ala	gct Ala 370		a ti	tg eu	atc Ile	gg G1	cg yA 3	at sp 75	ato Ile	gc Al	g a	atc []e	aat Asn	1637
V				tg t eu P	tt t he s	tct Ser	ccc Pro 385	gaa Glu	agt uVa	c a	tt le	tta Leu	ta 1 Ty 39		tt al	tco Sei	c ct	u :	tcg Ser	gca Ala 395	1685
		gg GT	a g y A	cc t la T	уr	acg Thr 400	aca Thr	CC	a ag	jc t er T	ac yr	gaç G1i 40!		g a	igc ser	cte Lei	g.go u A	g la	aat Asr 41(t aaa 1 Lys)	1733
ā	atg 4et	gt Va	g a 1 L	ys ı	tg eu 115	ttt Phe	atg Met	ct Le	g at u I	. – -	tg eu 20	gtg Va	9 gg	ia i	ctt Leu	tt Ph	t a e L 4	aa ys 25	gtç Va	g gag I Gli	1781
Ģ	gga Sly	tt Ph	e v			gga Gly	tta Lei	ac I Th		tc t le L 35	ta .eu	•••	. –			at Me 44	g a t T 0	ct hr	tc Se	g ato r Ilo	1829 e
			•									Pa	.ge	130							

agg tca tt Arg Ser Le 445	g cga acg cct u Arg Thr Pro	tac tta tgg Tyr Leu Trp 450	CCT CTC CTC Pro Leu Leu 455	ccg ttc aat gga Pro Phe Asn Gly	1877
aaa gcg tt Lys Ala Ph 460	t tgg cat gtt e Trp His Val 465	ctc gtg cgc Leu Val Arg	acg tcc gtt Thr Ser Val 470	cca ggg gga aaa Pro Gly Gly Lys 475	1925
gtc agg cc Val Arg Pr	g agc atc gtt o ser Ile Val 480	cat ccg aga His Pro Arg	aac cgc tcc Asn Arg Ser 485	aga cag ccg Arg Gln Pro 490	1970
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<213> Bacillus Ticheniformis

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Asp Leu Gly Val Arg Lys Val Phe Ile Leu Gly His Glu Val Gln Leu 35 40 45

Tyr Tyr Val Asn Gly Leu Cys Asp Thr Gln Tyr Ile Ile His Leu Leu 50 60

Arg Glu Leu Val His Leu Asn Asp Lys Glu Lys Glu Ser Gly Glu Val 65 70 75 80

Glu Asp Ile Val Glu Asn Arg Leu Leu Asn Gln Gln Val Ser Lys Ala 85 90 95 Glu Thr Leu Asp Glu Ala Val Asp Gln Val Leu Ser Gly Leu Val Ala 100 105 110 Ile Ile Val Glu Asp Ala Gly Phe Ala Phe Ile Ile Asp Val Arg Ser 115 120 125 Tyr Pro Gly Arg Thr Pro Glu Glu Pro Asp Thr Glu Lys Val Val Arg 130 135 140 Gly Ala Arg Asp Gly Leu Val Glu Asn Ile Ile Val Asn Thr Ala Leu 145 150 150 160 Ile Arg Arg Arg Ile Arg Asp Glu Arg Leu Arg Tyr Lys Met Leu His 165 170 175 Ile Gly Glu Arg Ser Lys Thr Asp Ile Cys Leu Cys Tyr Leu Glu Asp 180 185 Val Ala Asp Pro Asp Leu Val Glu Val Leu Lys Lys Glu Ile Glu Asp 195 200 205 Val Lys Ile Asp Gly Leu Pro Met Ser Asp Lys Ser Val Glu Glu Phe 210 215 220 Leu Val Gly Gln Gly Tyr Asn Pro Phe Pro Leu Val Arg Phe Thr Glu 225 230 235 240 Arg Ala Asp Val Ala Ala Ser His Ile Leu Glu Gly His Val Ile Val 245 250 255 Ile Val Asp Thr Ser Pro Ser Val Ile Ile Thr Pro Thr Thr Leu Phe 260 270 His His Val Gln His Ala Glu Glu Tyr Arg Gln Thr Pro Ala Val Gly
275 280 285 Thr Phe Leu Arg Trp Val Arg Phe Phe Gly Ile Leu Ala Ser Thr Phe 290 295 300 Leu Leu Pro Leu Trp Leu Leu Phe Val Ile His Pro Ser Leu Leu Pro 305 310 315 320 Asp Asn Leu Ser Phe Ile Gly Leu Asn Lys Asp Thr His Ile Pro Ile 325 330 335 Ile Met Gln Ile Phe Leu Ala Asp Leu Gly Val Glu Phe Leu Arg Met 340 350 Ala Ala Ile His Thr Pro Thr Ala Leu Ser Thr Ala Met Gly Leu Ile 355 360 365

Ala Ala Val Leu Ile Gly Asp Ile Ala Ile Asn Val Gly Leu Phe Ser 370 380

Pro Glu Val Ile Leu Tyr Val Ser Leu Ser Ala Ile Gly Ala Tyr Thr 385 390 395 400

Thr Pro Ser Tyr Glu Leu Ser Leu Ala Asn Lys Met Val Lys Leu Phe 405 410 415

Met Leu Ile Leu Val Ala Leu Phe Lys Val Glu Gly Phe Val Ile Gly 420 425 430

Leu Thr Ile Leu Thr Ile Val Met Thr Ser Ile Arg Ser Leu Arg Thr 435 440 445

Pro Tyr Leu Trp Pro Leu Leu Pro Phe Asn Gly Lys Ala Phe Trp His 450 460

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Val His Pro Arg Asn Arg Ser Arg Gln Pro 485 490

<210> 88

<211> 1567

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<223>

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taagggagg atatagaata atg ctt gtg ttt gca gga ttg ggc aat ccg ggt Met Leu Val Phe Ala Gly Leu Gly Asn Pro Gly 10	533
laa aca tat gaa aat acg aga cac aat gta ggt ttt atg acg att gac _ys Thr Tyr Glu Asn Thr Arg His Asn Val Gly Phe Met Thr Ile Asp _25 20 25	581
Jag ctc tcg aaa gag tgg aac att ccg ctt gat aaa aca aag ttc aac 31u Leu Ser Lys Glu Trp Asn Ile Pro Leu Asp Lys Thr Lys Phe Asn 30 35	629
gga caa tac gga atc ggg ttt gtt tcc ggc aaa aag gtt cta ctt gtt Gly Gln Tyr Gly Ile Gly Phe Val Ser Gly Lys Lys Val Leu Leu Val 45	677
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gat ttg gat ctt ccg acc gga aga atc cgt ctg agg acg aaa gga agc Asp Leu Asp Leu Pro Thr Gly Arg Ile Arg Leu Arg Thr Lys Gly Ser 95 100 105	821
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agt gag ttt aac cgg atc aga atc gga ata ggc cgt ccg gta aac ggc Ser Glu Phe Asn Arg Ile Arg Ile Gly Ile Gly Arg Pro Val Asn Gly 125 130	917
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<210> 89

<211> 188

<212> PRT

<213> Bacillus licheniformis

<400> 89

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Trp Asn Ile Pro Leu Asp Lys Thr Lys Phe Asn Gly Gln Tyr Gly Ile 35 40 45

Gly Phe Val Ser Gly Lys Lys Val Leu Leu Val Lys Pro Leu Thr Tyr 50 60

Met Asn Leu Ser Gly Glu Cys Leu Arg Pro Leu Leu Asp Tyr Tyr Glu 65 70 75 80

Ile Pro Val Asp Asn Leu Lys Val Ile Tyr Asp Asp Leu Asp Leu Pro 85 90 95

Thr Gly Arg Ile Arg Leu Arg Thr Lys Gly Ser Ala Gly Gly His Asn 100 105 110

Gly Ile Lys Ser Thr Ile Gln His Leu Gly Thr Ser Glu Phe Asn Arg 115 120 125

Ile Arg Ile Gly Ile Gly Arg Pro Val Asn Gly Met Lys Val Val Asp 130 140

Tyr Val Leu Gly Ala Phe Thr Asp Glu Glu Glu Pro Ala Ile Lys Glu 145 150 155 160

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222> (501)..(1598)

223>

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tgatgaacta aagccgcata tgtcttttgt aaaagcggtg atcactttcg gcgagaccgc	240
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ccc ggg atc ggc atg gaa cgg aac ggg tcg agg agc tgg atc gga gtc Pro Gly Ile Gly Met Glu Arg Asn Gly Ser Arg Ser Trp Ile Gly Val 95 100	821
ggc gct ttc agc att cag ccg tcc gag ttt atg aaa ctc gcg atg atc Gly Ala Phe Ser Ile Gln Pro Ser Glu Phe Met Lys Leu Ala Met Ile 110 115	869
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ttt aga aaa ggc ttt gtg ccg gcg ctg ggc att gtc ttt tca gct ttt Page 136	965

Phe Arg Lys Gly Phe Val Pro Ala Leu Gly Ile Val Phe Ser Ala Phe 140 145 150 155	
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<211> 366

<212> PRT

<213> Bacillus licheniformis

<400> 91

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35 40 45

Gln Leu Leu Phe Ala Gly Ile Gly Val Ile Ala Met Phe Phe Ile Met 50 60

Asn Val Asp Tyr Trp Thr Trp Arg Thr Tyr Ala Lys Ile Leu Ile Ile 65 70 75 80

Val Cys Phe Phe Leu Leu Ile Ile Val Leu Val Pro Gly Ile Gly Met 85 90 95

Glu Arg Asn Gly Ser Arg Ser Trp Ile Gly Val Gly Ala Phe Ser Ile 100 105 110

Gln Pro Ser Glu Phe Met Lys Leu Ala Met Ile Ala Phe Leu Ala Lys 115 120 125

Phe Leu Ser Glu Lys Gln Lys Asn Ile Thr Ser Phe Arg Lys Gly Phe 130 140

Val Pro Ala Leu Gly Ile Val Phe Ser Ala Phe Leu Ile Ile Met Met 145 150 155 160

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Met Ile Phe Val Ala Gly Ala Arg Ile Ser His Phe Val Phe Leu Gly 180 185 190

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<210> 92

<211> 1882

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agaaggtgaa tgtt	agagcc atg to Met Lo 1	ta acc gga eu Thr Gly	ttg acg at Leu Thr Il 5	t gca atc at e Ala Ile Il 10	e GIY
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tta gac ggc ato Leu Asp Gly Mei 110	gcc aaa ggg Ala Lys Gly	gcg aac c Ala Asn A 115	gt cgt ctt Arg Arg Leu	atc aag ctc Ile Lys Leu 120	ttt 869 Phe
gaa aga gac gat Glu Arg Asp Asp 125	att gcg att Ile Ala Ile 130	Tyr Asn S	ccg ata cct ser Ile Pro 135	aca gtc gaa Thr Val Glu	ggt 917 Gly
gcc att atg ato Ala Ile Met Mei 140	g gcc ata cag Ala Ile Gln 145	cat aca g His Thr A	ac ttt acg Asp Phe Thr 150	att cac ggc Ile His Gly	tcg 965 Ser 155
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Page 140

	1205
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cc ggg atc gtc gcg cct aaa acg gcg gga cag atc att gcc aat gtt ro Gly Ile Val Ala Pro Lys Thr Ala Gly Gln Ile Ile Ala Asn Val 270 275	1349
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Bacillus licheniformis <213>

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Lys Leu Asn Glu Leu Asp Phe Gly Thr Ile Asp Ser Ile Ile Leu Pro 50 60

'al Ser Gly Thr Ser Met Glu Gly Thr Val Ala Thr Val Phe Ser Asn 70 75 ilu Lys Val Val Leu Lys Gln Glu His Leu Glu Lys Thr Lys Pro His 90 95 Tys Ala Ile Tyr Ser Gly Ile Ser Asn Gln Tyr Leu Asp Gly Met Ala
100 105 Lys Gly Ala Asn Arg Arg Leu Ile Lys Leu Phe Glu Arg Asp Asp Ile 115 120 125 Ala ile Tyr Asn Ser ile Pro Thr Val Glu Gly Ala ile Met Met Ala 130 135 Ile Gln His Thr Asp Phe Thr Ile His Gly Ser Asn Val Met Val Leu 145 150 160 Gly Leu Gly Arg Thr Gly Met Ser Ile Ser Arg Thr Phe Ser Ala Leu 165 170 175 Gly Ala Arg Val Lys Val Gly Ala Arg Asp Ser Ala His Leu Ala Arg 180 185 Tle Met Glu Met Gly Leu Thr Pro Phe His Thr Asn Glu Leu Ala Glu 195 200 205 His Val Glu Asn Ile Asp Ile Cys Ile Asn Thr Ile Pro Ser Leu Ile 210 215 220 Leu Asp Lys His Val Leu Ser Arg Met Thr Pro Arg Thr Leu Ile Leu 225 230 235 Asp Leu Ala Thr Arg Pro Gly Gly Thr Asp Phe Asp Phe Ala Glu Lys 245 Gln Gly Ile Lys Ala Leu Leu Ala Pro Gly Leu Pro Gly Ile Val Ala 260 265 Pro Lys Thr Ala Gly Gln Ile Ile Ala Asn Val Leu Cys Asn Leu Leu 275 280 285 Ser Glu Leu Thr Thr Asp Arg Lys Gly Leu Ser 290 295

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the second and are are are all are are are all	533
gaccgaaagg ggctgtcata atg tcg atc ada gga ada agg tie gly Phe Gly Met Ser Ile Lys Gly Lys Arg Ile Gly Phe Gly 10	
cta acg ggt tca cat tgt acg tat gat gcc gtt ttt ccg cag att gaa Leu Thr Gly Ser His Cys Thr Tyr Asp Ala Val Phe Pro Gln Ile Glu	581
15	629
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30 33	677
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45 50 33	725
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60 65	
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80	
cca ttg acg gga aat tcg atg agc aag ctt gca aac gcc cag acg gac	821
Pro Leu Thr Gly Asn Ser Met Ser Lys Leu Ala Asn Ala Gln Thr Asp 95 100 105	
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ser Pro val Leu Met Ala Ala Lys Ala Thr Met Arg Asn Ser Arg Pro 110 115	
gtc gtc ctc ggc att tca acg aat gac gcg ctc ggc ttg aac ggc gtc	917
Val Val Leu Gly Ile Ser Thr Asn Asp Ala Leu Gly Leu Asn Gly Val 125	
aac ttg atg agg ctg atg gcg gca aaa aat gtt tac ttt att ccg ttc	965
Ash Leu Met Arg Leu Met Ara Ara Lys Ash va. 177 178 155	
Page 143	

10293.5123.1%	
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cag cct atc ctg gtc cat aat gat caa taaatctttt gaaaataaag Gln Pro Ile Leu Val His Asn Asp Gln 190 195	1108
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Thr Arg Phe Gly Glu Glu Glu Trp Val Arg Arg Ile Glu Glu Leu 50 60	
Thr Gly Phe Glu Val Ile Asp Ser Ile Pro Lys Ala Glu Pro Leu Gly 65 75 80	
Pro Lys Thr Pro Leu Asp Cys Met Val Val Ala Pro Leu Thr Gly Asm 85 90 95	
Ser Met Ser Lys Leu Ala Asn Ala Gln Thr Asp Ser Pro Val Leu Met 100 105 110	

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Ser Thr Asn Asp Ala Leu Gly Leu Asn Gly Val Asn Leu Met Arg Leu 130 135 140	
Met Ala Ala Lys Asn Val Tyr Phe Ile Pro Phe Gly Gln Asp Asp Pro 145 150 150	
Tyr Lys Lys Pro Asn Ser Leu Val Ala Lys Met Asp Leu Leu Val Pro 175 165	
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Glu	Glu	Ser	17e 15	Cys	Phe	Gln (Lys	102 Gly (20	95.S Gln	T25. Glu	txt Val	Ser (G]u 25	Leu	Leu	
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gta Val	tcc ser 45	ata Ile	cga Arg	ggg Gly	tca Ser	tta Leu 50	gag Glu	ctg Leu	aca Thr	ggc Gly	gaa Glu 55	tac Tyr	aac Asn	ata Ile	gat Asp	677
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gaa Glu	ace Thi	r va	t cc	g ati	tac Tyr	cag Glr 210) Sei	ttt Phe	cto Lei	gga i Gly	a aad / Asr 215	1 426	aca Thr	gag Gli	gaa Glu	1157
gc1 A1a 220	Ly:	a cc s Pr	g tt o Ph	t tti e Pho	t aca e Thi 22!	CAI	tct Ser	ttg Lei	tco Sei	g gcg r A1a 230	a Ale	a gag a Gli	g cgt u Arg	acg Thi	aag Lys 235	1205
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ga: Gl:	a ta u Ty	t aa r Ly	g ct s Le 25	u Ly	a aga s Ar	a gaç g Gli	g aaa u Ly:	a gtg s Va 260	<u>.</u> G:	a ga u Gl	g gaa u Gl	a cce u Pre	g gaa o G1: 26:		a tat u Tyr	1301
ga Gl	g ct u Le	g aa u Ly 27	a ag s Ar	a ga g G1	g aa u Ly	a gte s va	g ga: I G1: 27	a gaq u Gli 5	g ga u Gl	a cc u Pr	g ga o Gl	a ga u G1 28	a ta u Ty 0	t ga r Gl	g ctg u Leu	1349
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cat His	acg Thr	att Ile 350	gaa Glu	atc Ile	ccg Pro	gaa Glu	tat Tyr 355	tcg Ser	ttt Phe	cat His	gag Glu	cag Gln 360	* * * * * * * * * * * * * * * * * * * *	gag Glu	CCC Pro	1589
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gga Gly	cag Glr 445	ıle	ctt Lei	tat Tyr	ata Ile	ccg Pro 450) AS	t tat Tyr	caa Glr	aae Asi	ago 1 Sei 45!	" HIS	gco s Ala	i i		1871
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Glu Glu Glu Pro Glu Leu Ser His Ser Ser Tyr Gln Pro His Glu Glu 290 295 300 Leu Lys Glu Asn Pro Phe Tyr Ser Val Pro Pro Leu Leu Lys Glu Asp 305 310 315 Gln Asn Asp Arg Glu Pro Glu Ala Phe Glu Val Glu Val Thr Gln Glu 325 330 335 Ala Glu Ala Ile Asp Glu Glu Glu Glu Ala Gly His Thr Ile Glu Ile 340 350 Pro Glu Tyr Ser Phe His Glu Gln Thr Glu Pro Glu Glu Glu Arg Asp 355 360 365 Glu Met Gln Ala Ala Asp Glu Gln Glu Val Ser Ala Lys Glu Asn Asp 370 380 Asn Ala Leu Tyr Leu Thr Lys Leu Phe Thr Lys Gln Gly Glu Glu 385 390 395 Phe Thr Arg Met Arg Met Cys Ile Val Gln Gln Asn Asp Thr Ile Asp 405 410 Leu Leu Cys Glu Arg Tyr Asp Ile Asn Val Gln Gln Leu Ile Arg Met 420 430 Asn Ser Leu Ser Leu Asp Glu Glu Leu Lys Glu Gly Gln Ile Leu Tyr 435 440 445 Ile Pro Asp Tyr Gln Asn Ser His Ala 450

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<223>

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Page 154

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Arg Glu Asn Ala Ala Met Lys Gln Gln Leu Gln Gln Leu Ser Phe Glu 50 60

Leu Glu Arg Ile Ser Ala Asn Lys Glu Asp Lys Ser Ala Glu Thr Leu 65 70 75 80

Asn Arg Ile Lys Ser Glu Leu Leu Ser Lys Ile Val Val Leu Gln Glu 85 90 95

Leu Leu Gln Lys Glu Thr Tyr Ala Arg Lys Gln Glu Ile Glu Glu Lys 100 110

His Arg Leu His Leu Thr Asn Val Lys Ala Glu Glu Lys Lys Ser 115 120 125

Leu His Ser Gln Ile Glu Tyr Glu Lys Leu His Ala Glu Arg Glu Lys 130 135 140

Thr Leu Arg Glu Lys Lys Glu Gln Glu Leu Lys Asn Ala Ala Tyr Glu 145 150 155 160 Asn Ala Arg Leu Lys Asp Glu Leu His Ala Lys Ser Leu Gln Leu Lys 165 170 175

Gln Ile Glu Thr Asp Val Ala Val Leu Lys Glu Arg Val Thr Glu Thr 180 185 190

Lys Ser Arg Leu Leu Glu Ala Glu Lys Thr Lys Glu Ala Leu Phe Tyr 195 200 205

Glu Thr Ile Leu Ser Tyr Lys Arg Gln Leu Asp Glu Ser Asp Lys Trp 210 215 220

Ile Ala Ser His Phe Ala Asp Ile Asp Ala Phe Gln Gln Thr Glu Lys 235 230 235

Ala Leu Glu Gln Asn Glu Glu Val Phe Glu Arg Thr Glu Gln Ile Glu 245 250 255

Ala Val Leu Gln Thr Val Thr Glu Gln Val Asp Gln Leu Gln Gln Gln 260 265 . 270

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aagaagcggt agatcaaaat aaaaaagaaa cagaagcttt attttctat aatcccgaca 240
Page 174

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gaa agc atg Glu Ser Met						773
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act tct ctt of the ser Leu i						.061
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			Page 175			

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gct ggc gta Ala Gly Val	tca aga aca Ser Arg Thr 255	Ser Ala	atc gac Ile Asp 260	cag tto Gln Pho	ggc atg Gly Met 265	ctt tcg Leu Ser	1301
gga gtt gcg Gly Val Ala 270	atg aca atc Met Thr Ile	ggc ttt i Gly Phe i 275	ttt ccg Phe Pro	gct tti Ala Phe	atc gcc lle Ala 280	cat tca His Ser	1349
ctg atg gtc Leu Met Val 285	gtc atg atc Val Met Ile	ccg agc a Pro Ser 1 290	att tct Ile Ser	gaa ago Glu Sei 295	Tyr Āla	tac ggg Tyr Gly	1397
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ctg ttt tac Leu Phe Tyr	ggc ata ccg Gly Ile Pro 320	tcc gtc a Ser Val R	atg gtg Met Val 325	atg ta Met Tyl	cac ttt His Phe	gca gag Ala Glu 330	1493
ccg ctg acc Pro Leu Thr		Phe Asp §					1541
aaa atg ttg Lys Met Leu 350	tgg ccg tat Trp Pro Tyr	ttt tta t Phe Leu F 355	ttc cac Phe His	ttt tti Phe Phe	gcg atg Ala Met 360	cct ttt Pro Phe	1589
cag gcc tgt Gln Ala Cys 365	tta atc gga Leu Ile Gly	atg ggg t Met Gly i 370	ttg gcc Leu Ala	aaa gat Lys Asp 37) Ala Phe	tat cat Tyr His	1637
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cgattccaat co	•						2178
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2298 2341

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Phe Leu Val Val Ile Ala Ser Leu Glu Leu Pro Val Ser Ile Ser 50 60

Lys Phe Ile Ala Glu Ser Asn Pro Lys Leu His Glu Ser Met Leu Lys 70 75 80

His Ala Leu Arg Met Thr Ala Val Cys Thr Val Phe Ser Thr Ala Ala 85 90 95

Ala Val Ile Ile Leu Pro Phe Ile Pro Val Phe Asp Ser Tyr His Pro 100 105 110

Leu Ile Arg Gly Leu Val Ile Gly Met Ile Pro Thr Val Ala Phe Thr 115 120 125

Ser Ile Ala Arg Gly Tyr Phe Met Gly Val Gln Gln Met Gly Lys Ile 130 140

Ala Thr Ala Asn Ala Leu Lys Lys Ile Phe Gln Leu Ile Gly Leu Phe 145 150 155 160

Leu Phe Phe Gln Trp Tyr Ser Phe Glu Leu Asp Thr Ser Leu Leu Ile 165 170 175

Ser Leu Phe Val Leu Val Ala Ser Glu Val Val Val Phe Val Tyr Leu 180 185 190

Phe Ser Gln Phe Val Leu Val Arg Arg Ala Ala Gln Lys Gly Gln Gln 195 200 205 Page 177

Ile His Leu Arg Arg Asn Asp Val Leu Lys Arg Leu Leu Thr Val Ser 210 220 Ile Pro Thr Thr Gly Leu Arg Val Phe His Ala Val Thr Asn Ala Val 225 230 235 240 Glu Pro Phe Leu Val Lys Gly Thr Leu Leu Ala Gly Val Ser Arg 245 250 255 Thr Ser Ala Ile Asp Gln Phe Gly Met Leu Ser Gly Val Ala Met Thr 260 265 270 Ile Gly Phe Phe Pro Ala Phe Ile Ala His Ser Leu Met Val Val Met 275 280 285 Ile Pro Ser Ile Ser Glu Ser Tyr Ala Tyr Gly Gln Tyr Glu Arg Val 290 295 300 Ile Lys Arg Ile Lys Gln Ala Ile Phe Ile Thr Leu Phe Tyr Gly Ile 305 310 315 320 Pro Ser Val Met Val Met Tyr His Phe Ala Glu Pro Leu Thr His Leu 325 330 335 Phe Phe Asp Ser Val Lys Ala Ser Phe Tyr Leu Lys Met Leu Trp Pro 340 345 Tyr Phe Leu Phe His Phe Phe Ala Met Pro Phe Gln Ala Cys Leu Ile 355 360 365 Gly Met Gly Leu Ala Lys Asp Ala Phe Tyr His Asn Val Trp Ala Ser 370 380 Val Leu Ser Phe Leu Met Met Tyr Val Leu Gly Ser Met Gln Thr Leu 385 390 395 400 Gln Met Thr Gly Ile Ile Leu Ala Met Asn Thr Gly Met Ile Leu Leu 405 415 Thr Ala Leu His Tyr Val Thr Ile Cys Lys Glu Leu Gly Val Thr Leu 420 425 430 Phe Leu Thr Asn Lys Ser Arg Ser Pro Arg Ile Glu Ser Arg 445

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rage 1/3	

10233.3123.000
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50 55 60
Ile Leu Thr Val Tyr Ala Leu Lys His Val Ser Ile Glu Asn Arg Gly
65 70 75 80
Gly Val Leu Tyr Phe Arg Thr His Leu Trp Val Glu Leu Ile Val Leu
85 90 95
The state of the same of the s
Phe Leu Phe Leu Tyr Arg Phe Leu Tyr Arg Ile Ala Glu Ile Gly Gln 100 105 110
Leu Gln Thr Ala Val Ser Asp Gly Gly Ser Ala Ala Tyr Gly Ala Leu 115 120 125
Phe Ala Gin Asp Pro Ala Thr Met Ile Gly Phe Phe Val Leu Ala Val 130 135 140
Page 180

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gtc tgg aaa acg act tct gaa gcg cat aac aat gtc agc cag ctg ccg

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725

773

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Bacillus licheniformis

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Asp Val Trp Leu Tyr Pro Asp Ser Ile Arg Ile Lys Val Ala Leu Asp 340 350

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•	Val Gln									Thr						
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Page 189

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Thr Ala Val Tyr Asp Ser Ala Glu Leu Lys Lys Glu Ile Gly Arg Leu 85 90 95

Thr Glu Cys Phe Pro Phe Val Thr Ser Arg Ile Ile Gly Arg Ser Ser 100 105 110

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Lys Arg Thr His Met Asn Ala Ser Phe His Ala Asn Glu Trp Ile Thr 130 140

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Arg Ala Lys Thr Tyr Asn Val Pro Lys Ala Ile Ile Glu Arg Ala Ile 65 70 75

Glu Lys Ala Lys Gly Gly Ser Glu Glu Asn Tyr Asp Glu Leu Arg Tyr 85 90 95 Page 194

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Tyr Val Ile Arg Met Val Ser Leu Ala Leu Leu Val Val Pro Ile Leu
50 55 191 gcg ctg atc aga ggc ttt ttc caa ggt cac cag atg atg ggg ccg aca Ala Leu Ile Arg Gly Phe Phe Gln Gly His Gln Met Met Gly Pro Thr 65 70 75 239 gcc gtt tca cag gta gtt gaa caa att gcc aga atc gtc ttt cta tta Ala Val Ser Gln Val Val Glu Gln Ile Ala Arg Ile Val Phe Leu Leu 80 85 287 acg gcc act tac ttg gtg atc aaa gta tta aac ggc ggg ctt gtc gtc Thr Ala Thr Tyr Leu Val Ile Lys Val Leu Asn Gly Gly Leu Val Val 100 105 335 gct gtc ggc tat gcg act ttt gcg gct ttg atc gga gcg ttc gcc gga Ala Val Gly Tyr Ala Thr Phe Ala Ala Leu Ile Gly Ala Phe Ala Gly 115 120 125 383 ctg ttc act ctt tac ttt tcc tgg cag aaa aga aaa ggg gcg ctc ctg Leu Phe Thr Leu Tyr Phe Ser Trp Gln Lys Arg Lys Gly Ala Leu Leu 130 431 gcg ctg aag ccg aac ctt gtt cct tca gcc gat att acg tac cgg caa Ala Leu Lys Pro Asn Leu Val Pro Ser Ala Asp Ile Thr Tyr Arg Gln 145 150 479 atg ttt aaa gag ctg ttc agc tat gcc gcc cct tat gtc ttt gtc ggg Met Phe Lys Glu Leu Phe Ser Tyr Ala Ala Pro Tyr Val Phe Val Gly 160 165 170 527 ctg gcg ata ccg ctt tac cag tac att gat acg aat acg ttt aat aaa Leu Ala Ile Pro Leu Tyr Gln Tyr Ile Asp Thr Asn Thr Phe Asn Lys 180 575 gcg atg att gca gcc ggc tat caa aac atc agc cag gat ttg atg gcg Ala Met Ile Ala Ala Gly Tyr Gln Asn Ile Ser Gln Asp Leu Met Ala 195 200 205 623 acg ctg tac gtg cca aag ctt gtg atg att ccg gta tct ctc Thr Leu Tyr Val Pro Lys Leu Val Met Ile Pro Val Ser Leu 210 220 671 gcg acg gca ttc ggg ctg aca ttg att ccg gcg gtg act gaa aac ttt Ala Thr Ala Phe Gly Leu Thr Leu Ile Pro Ala Val Thr Glu Asn Phe 225 230 235 719 - acc-aac aaa gat ttc cct gct tta aac aaa cag att gat cag gcg atg Thr Asn Lys Asp Phe Pro Ala Leu Asn Lys Gln-Ile Asp Gln Ala Met 240 245 767 cag atc att ctc ttc atc gtt ctt ccg gca tca gtc ggt atg gct ctt Gln Ile Ile Leu Phe Ile Val Leu Pro Ala Ser Val Gly Met Ala Leu 260 265 270 815 ttg tcg ggg ccg gtt tac acg ttc ttt tac ggc tcg gaa agc ctg ctc Leu Ser Gly Pro Val Tyr Thr Phe Phe Tyr Gly Ser Glu Ser Leu Leu 285 863 cct gac atg gga cga gat att ttg ttc tgg tac gcg cct gtg gcg ctg 911 Page 196

Pro Asp Met Gly Arg Asp Ile Leu Phe Trp Tyr Ala Pro Val Ala Leu 290 295 300	
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<213> Bacillus licheniformis

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Leu Gly Gly Thr Glu Asn Asn Gly Leu Thr Leu Glu His Val Val Tyr 35 40 45

Val Ile Arg Met Val Ser Leu Ala Leu Leu Val Val Pro Ile Leu Ala 50 60

Leu Ile Arg Gly Phe Phe Gln Gly His Gln Met Met Gly Pro Thr Ala 80

Val Ser Gln Val Val Glu Gln Ile Ala Arg Ile Val Phe Leu Leu Thr 85 90 95

Ala Thr Tyr Leu Val Ile Lys Val Leu Asn Gly Gly Leu Val Val Ala 100 105 110

Val Gly Tyr Ala Thr Phe Ala Ala Leu Ile Gly Ala Phe Ala Gly Leu 125 120

Phe Thr Leu Tyr Phe Ser Trp Gln Lys Arg Lys Gly Ala Leu Leu Ala 130 135

Leu Lys Pro Asn Leu Val Pro Ser Ala Asp Ile Thr Tyr Arg Gln Met 145 150 160

Phe Lys Glu Leu Phe Ser Tyr Ala Ala Pro Tyr Val Phe Val Gly Leu 165 170

Ala Ile Pro Leu Tyr Gln Tyr Ile Asp Thr Asn Thr Phe Asn Lys Ala 180 185

Met Ile Ala Ala Gly Tyr Gln Asn Ile Ser Gln Asp Leu Met Ala Ile 200 205

Val Thr Leu Tyr Val Pro Lys Leu Val Met Ile Pro Val Ser Leu Ala 210 220

Thr Ala Phe Gly Leu Thr Leu Ile Pro Ala Val Thr Glu Asn Phe Thr 225 230 235

Asn Lys Asp Phe Pro Ala Leu Asn Lys Gln Ile Asp Gln Ala Met Gln 250 255 Page 198

Ile Ile Leu Phe Ile Val Leu Pro Ala Ser Val Gly Met Ala Leu Leu 260 265 Ser Gly Pro Val Tyr Thr Phe Phe Tyr Gly Ser Glu Ser Leu Leu Pro 275 280 285 Asp Met Gly Arg Asp Ile Leu Phe Trp Tyr Ala Pro Val Ala Leu Leu 290 300 Phe Ser Leu Phe Thr Val Asn Ala Ala Ile Leu Gln Gly Val Asn Lys 315 Gln Lys Phe Ala Val Val Ser Leu Met Ile Gly Ile Val Ile Lys Ile 325 Ala Leu Asn Val Pro Leu Ile Lys Leu Leu Gln Gly Ser Gly Ser Ile 340 345 Leu Ala Thr Ala Leu Gly Tyr Ser Ala Ser Leu Leu Tyr Gly Phe Ile 355 360 365 Met Ile Lys Arg His Ala Gly Tyr Ser Tyr Arg Lys Leu Phe Lys Arg 370 380 Phe Leu Leu Met Leu Ile Leu Thr Ala Val Met Gly Ile Ile Leu Leu 385 390 395 Leu Val Gln Ala Leu Leu Ser Ile Phe Ile Ser Tyr Glu Gly Gly Gln 405 410 Ile Arg Ser Ala Val Val Ile Phe Ile Thr Thr Ala Val Gly Gly Ser 420 425 430

Val Tyr Leu Tyr Leu Ala Tyr Arg Val Lys Leu Leu Glu Lys Ile Phe 445

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<211> 1852

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<213> Bacillus licheniformis

<220>

<221> CDS

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cggtttattc gtagcattaa aagccgcttc ggctgatgaa gaaattgaaa cgggcaaaaa	240
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aatttcgtga tgtcacagaa ggaaaattca tgagaaaata gaattataaa aatggcagtg	480
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gaa gaa att caa gag att cca gta ggc gat ata att cct aac cgt ttt Glu Glu Ile Gln Glu Ile Pro Val Gly Asp Ile Ile Pro Asn Arg Phe 30 35 40	629
cag ccg cgc acc att ttc tca gaa gaa aaa att aaa gaa tta gct gca Gln Pro Arg Thr Ile Phe Ser Glu Glu Lys Ile Lys Glu Leu Ala Ala 45 50 55	677
acc att cat aca cac ggc att atc cag ccg att gtc gtc aga aaa aca Thr Ile His Thr His Gly Ile Ile Gln Pro Ile Val Val Arg Lys Thr 60 65 70 75	725
gag cgg gaa ggc caa tat gaa ctc ata gcc gga gag cgg cgc tgg cgg Glu Arg Glu Gly Gln Tyr Glu Leu Ile Ala Gly Glu Arg Arg Trp Arg 80 85 90	773
gcg gtt caa acg ctc gat tgg gag aag gtt ccc gct att att aag gat Ala Val Gln Thr Leu Asp Trp Glu Lys Val Pro Ala Ile Ile Lys Asp 95 100 105	821
ttt tca gat aca gag acc gct tct gtc gct ctt atc gaa aac ctt cag Phe Ser Asp Thr Glu Thr Ala Ser Val Ala Leu Ile Glu Asn Leu Gln 110 115 120	869
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tta gag ctt cac gat ttg acg cag gaa gcc ctt gca caa agg ctt gga Leu Glu Leu His Asp Leu Thr Gln Glu Ala Leu Ala Gln Arg Leu Gly 140 145 150 155	965
aag ggc cag tca aca atc gcc aat aag ctc aga ctg tta aag ctt ccg Lys Gly Gln Ser Thr Ile Ala Asn Lys Leu Arg Leu Leu Lys Leu Pro 160 165 170	1013
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10295.ST25.txt	
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ctg cat gaa gtc att gaa aag agt tta aat gta aaa caa acc gaa gac Leu His Glu Val Ile Glu Lys Ser Leu Asn Val Lys Gln Thr Glu Asp 205 210 215	1157
cgt gtc gtc aaa atg ctt gag cag gat aaa cgc aag cct aaa cca aag Arg Val Val Lys Met Leu Glu Gln Asp Lys Arg Lys Pro Lys Pro Lys 225 230 235	1205
aga aaa gcg tac agc agg gac gcg aga atc gcg atg aat acg att cgc Arg Lys Ala Tyr Ser Arg Asp Ala Arg Ile Ala Met Asn Thr Ile Arg 240 245 250	1253
cag tcc tta tca atg gtg gaa gac agc ggc gtc aaa ctg aat acg gaa Gln Ser Leu Ser Met Val Glu Asp Ser Gly Val Lys Leu Asn Thr Glu 265 265	1301
gaa gag gaa ttt gaa gaa tat att cag ttt acg att cga ata ccg aaa Glu Glu Glu Phe Glu Glu Tyr Ile Gln Phe Thr Ile Arg Ile Pro Lys 270 275	1349
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<211> 283

<212> PRT

<213> Bacillus licheniformis

<400> 129

Met Lys His Ser Phe Ser Arg Leu Phe Gly Leu Gly Asp Lys Glu Glu 10 15

Glu Ala Glu Ile Ala Glu His Asp Thr Asn Lys Glu Glu Ile Gln Glu 20 25 30

Ile Pro Val Gly Asp Ile Ile Pro Asn Arg Phe Gln Pro Arg Thr Ile 35 40 45 Page 201

Phe Ser Glu Glu Lys Ile Lys Glu Leu Ala Ala Thr Ile His Thr His 50 60 Gly Ile Ile Gln Pro Ile Val Val Arg Lys Thr Glu Arg Glu Gly Gln 65 70 75 Tyr Glu Leu Ile Ala Gly Glu Arg Arg Trp Arg Ala Val Gln Thr Leu 85 90 95 Asp Trp Glu Lys Val Pro Ala Ile Ile Lys Asp Phe Ser Asp Thr Glu 100 105 Thr Ala Ser Val Ala Leu Ile Glu Asn Leu Gln Arg Glu Glu Leu Ser 115 120 125 Ser Ile Glu Glu Ala His Ala Tyr Ala Arg Leu Leu Glu Leu His Asp 130 135 140 Leu Thr Gln Glu Ala Leu Ala Gln Arg Leu Gly Lys Gly Gln Ser Thr 145 150 160 Ile Ala Asn Lys Leu Arg Leu Leu Lys Leu Pro Glu Glu Val Gln Glu 165 170 175 Ala Ile Leu Lys Lys Glu Ile Ser Glu Arg His Ala Arg Ala Leu Ile 180 185 190 Pro Leu Lys Gln Pro Asp Leu Gln Val Lys Leu Leu His Glu Val Ile 195 200 205 Glu Lys Ser Leu Asn Val Lys Gln Thr Glu Asp Arg Val Val Lys Met 210 215 220 Leu Glu Gln Asp Lys Arg Lys Pro Lys Pro Lys Arg Lys Ala Tyr Ser 225 230 235 240 Arg Asp Ala Arg Ile Ala Met Asn Thr Ile Arg Gln Ser Leu Ser Met 245 250 255 Val Glu Asp Ser Gly Val Lys Leu Asn Thr Glu Glu Glu Phe Glu 260 265 270 Glu Tyr Ile Gln Phe Thr Ile Arg Ile Pro Lys 275